(54) Title: POLYNUCLEOTIDES AND POLYPEPTIDES OF AVIAN LEPTIN-RECEPTOR

(57) Abstract: Polynucleotide and polypeptide sequences of avian leptin-receptors and uses thereof.

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POLYNUCLEOTIDES AND POLYPEPTIDES OF AVIAN LEPTIN-RECEPTOR

5   FIELD AND BACKGROUND OF THE INVENTION

The present invention relates to polynucleotides encoding leptin-receptors and to polypeptides encoded by these polynucleotides and, more particularly, to polynucleotides encoding avian leptin-receptors and to polypeptides encoded by these polynucleotides.

10   *Leptins and leptin-receptors in mammals:*

The satiety hormone leptin and leptin-receptor (LR) are key regulators in the control of food intake, glucose metabolism, fat metabolism, energy expenditure, puberty, fertility, and immunity in mammals (reviewed in, Campfield *et al.*, 1996; Tartaglia, 1997; Auwerx

15   and Steels, 1998; Freidman and Halaas, 1998).

The circulating hormone leptin is produced primarily in the adipose tissues and signals to the hypothalamus indications relating to energy reserves. The gene encoding leptin was identified by positional cloning in 1994 (Zhang *et al.*, 1994) in a mouse homozygote for a Mendelian

20   segregating recessive mutation (*ob*) resulting, in its homozygous state (*ob/ob*), in a profound obese phenotype, including three times higher weigh than normal mice, five fold increase in body fat content, diabetes, infertility, low body temperature, low activity and hampered immunity.

The mouse and human leptin-receptor genes were discovered in 1995

25   (Tartaglia, *et al*, 1995; Luoh *et al.*, 1997, respectively) and led to the cloning of the leptin-receptor gene (or partial sequences thereof) in other mammalian species, including, rat, pig, sheep and cow. The mammalian leptin-receptor mediates the weight regulatory effects of its ligand, leptin, through interactions with a cytoplasmic kinase.

30   It was thereafter found that exogenous administration of recombinant leptin to *ob/ob* mice leads to a complete normalization of all aspects of the

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ob/ob phenotype (see, for example, Ahima *et al.*, 1997; Halaas *et al.*, 1997; Kamohara *et al.*, 1997; and Seeley *et al.*, 1996). The expression and secretion of leptin in mammals was shown to be highly correlated with body fat mass and adipocyte size.

5   At least six alternatively spliced leptin-receptor transcripts were identified in mammals designated Ob-Ra, Ob-Rb, Ob-Rc, Ob-Rd, and Ob-Re (Tartglia *et al.*, 1996; Lee *et al.*, 1998). Among these forms of the receptor, only the long variant (Ob-Rb) contains the cytoplasmic domain that interacts with the Jak kinase (JAK), and the signal transducer and

10   activator of transcription (STAT) proteins, which have been implicated with signal transduction of the leptin-receptor pathway (White *et al.*, 1997).

OB-Rb is expressed primarily in the paraventricular (VPN), ventromedial (VMH), dorsomedial (DMH), lateral (LN) and arcuate neurons of the hypothalamus - regions known to be associated with body

15   weight regulation (Hetherington and Ranson 1942; Mercer *et al.*, 1996; Fei *et al.*, 1997).

In the obese diabetic (*db/db*) mouse, a mutation is found in the OB-Rb form of the receptor, leading to a phenotype of extreme obesity and infertility, nearly indistinguishable from the phenotype of *ob/ob* mice.

20   These obesity mutations (*db* and *ob*) demonstrate a very unique situation in which a single protein and its related receptor play a key role in the two most agriculturally important parameters - feeding and fertility.

Implications to poultry industry:

While evidence for the therapeutic value of leptin and leptin-receptor

25   in humans accumulate (Friedman and Halaas, 1998), it still remains unclear whether this unique control mechanism is conserved in non-mammalian vertebrates, such as avians, as well.

Chicken, turkey and other poultry are the main source of protein for human consumption and as such, commercial poultry live-stocks, have been

30   genetically selected for fast growth.

As a result of such selection, broilers, for example, tend to over eat, which results in wastage of food and undesired accumulation of fat. Among broiler breeders, the problem of over eating, fat deposition and loss of fertility imposes a need to restrict feed intake by artificial means.

The situation is quite different for farm turkeys which accumulate insufficient amounts of fat in their carcass. It is believed that increasing the appetite of turkeys could serve both to improve fat deposition and to enhance growth rates.

In geese, for example, the need of force-feeding for the production of high quality *fois-gras* involves highly qualified manpower and evokes public objection, which is the biggest threat to this branch of the poultry industry in some areas of the Globe.

The ostrich branch of poultry is much less developed, yet seems to be of high future commercial potential.

Many of the problems listed hereinabove with respect to poultry breeding could be improved by controlling food consumption, energy expenditure and the like. Future tools to enhance puberty and improve fertility are important for growers of laying hens, turkeys, ostriches, gees and other commercially grown poultry.

Some indirect experimental results suggest similarities between the mechanisms regulating appetite and other aspects of energy balance among mammals and avians, no direct evidence has so far been provided in this respect (Lepkovsky and Yasuda, 1966; Snapir *et al.*, 1973; Kuenzel and Helms, 1967, Kuenzel *et al.*, 1987; Richardson *et al.*, 1995, Kuenzel and McMurty, 1998, Fraley and Kuenzel, 1993).

There is thus a widely recognized need for, and it would be highly advantageous to know whether (i) avians posses a mechanism for regulating appetite and other aspects of energy balance, such as, but not limited to, food consumption, energy expenditure, puberty, fertility and immunity, which is similar to that found in mammals, and if so, (ii) to clone the genes

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encoding the protein associated with such a mechanism; and (iii) to use such cloned genes to control appetite and other aspects of energy balance in avians, commercially bred poultry in particular.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a nucleic acid sequence encoding an avian, poultry in particular, leptin-receptor.

It is a further object of the present invention to provide a polynucleotide encoded by the avian leptin-receptor nucleic acid.

It is a further object of the present invention to provide an assay which will enable screening for leptin agonists and antagonists.

It is a further object of the present invention to provide for the ability to attenuate leptin activity in vivo, to thereby manipulate food intake, fat deposition, puberty and fertility in poultry.

It is a further object of the present invention to use the nucleic acid and polypeptide according to the present invention to isolate an avian leptin gene.

While reducing the present invention to practice, the chicken leptin-receptor gene was cloned, analyzed and sequenced. Cloning the chicken leptin-receptor gene represents the first non-mammalian derived isolated leptin-receptor gene.

Thus, according to one aspect of the present invention there is provided an isolated polynucleotide comprising a nucleic acid sequence at least 70 % identical with a nucleic acid encoding a polypeptide as set forth in SEQ ID NO:28 or a portion thereof longer than 30 contiguous amino acids, as determined using the Smith-Waterman algorithm and the following parameters: Matrix = Blosum62, gapopen = 10.0 and gapextend = 0.5.

According to another aspect of the present invention there is provided an isolated polynucleotide comprising a nucleic acid sequence hybridizable with a nucleic acid encoding a polypeptide as set forth in SEQ

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ID NO:28 under a hybridization selected from the group consisting of stringent hybridization and moderate hybridization, wherein stringent hybridization is effected by a hybridization solution of 6 x SSC, 5 x Denhardt's solution, 1 % SDS and 100 µg/ml denatured salmon sperm DNA, hybridization temperature of 65 °C, final wash solution of 0.1 x SSC and final at 60 °C, whereas moderate hybridization is effected by a hybridization solution of 6 x SSC, 5 x Denhardt's solution, 1 % SDS and 100 µg/ml denatured salmon sperm DNA, hybridization temperature of 58 °C, final wash solution of 0.5 x SSC and final wash at 50 °C.

According to further features in preferred embodiments of the invention described below, the nucleic acid sequence is as set forth in SEQ ID NO:27 or a portion thereof longer than 90 contiguous nucleotides and natural and man induced variants thereof.

According to still further features in the described preferred embodiments the nucleic acid sequence is of an avian.

According to still further features in the described preferred embodiments the polypeptide is capable of binding leptin under physiological conditions.

According to still further features in the described preferred embodiments the nucleic acid sequence is as set forth in SEQ ID NO:27.

According to still further features in the described preferred embodiments the isolated polynucleotide further comprising a vector ligated to the nucleic acid sequence.

According to still further features in the described preferred embodiments the nucleic acid sequence is a product of a polymerase chain reaction.

According to yet another aspect of the present invention there is provided a recombinant protein comprising an amino acid sequence at least 70, at least 80, at least 90 or at least 90-100 % homologous (identical +

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similar amino acids) with SEQ ID NO:28 or a portion thereof longer than 30 contiguous amino acids, as determined using the Smith-Waterman algorithm and the following parameters: Matrix = Blosum62, gapopen = 10.0 and gapextend = 0.5.

According to further features in preferred embodiments of the invention described below, the amino acid sequence is as set forth in SEQ ID NO:28 and natural and man induced variants thereof.

According to still further features in the described preferred embodiments the amino acid sequence is of an avian.

According to still further features in the described preferred embodiments the polypeptide is capable of binding leptin under physiological conditions.

According to still further features in the described preferred embodiments the amino acid sequence is as set forth in SEQ ID NO:28.

According to still another aspect of the present invention there is provided a single stranded polynucleotide of at least 18 nucleotides hybridizing with a nucleic acid sequence as set forth in SEQ ID NO:27 under a hybridization selected from the group consisting of stringent hybridization, moderate hybridization and mild hybridization, wherein stringent hybridization is effected by a hybridization solution of 6 x SSC and 1 % SDS or 3 M TMACI, 0.01 M sodium phosphate (pH 6.8), 1 mM EDTA (pH 7.6), 0.5 % SDS, 100 g/ml denatured salmon sperm DNA and 0.1 % nonfat dried milk, hybridization temperature of 1 - 1.5 °C below the T_m, final wash solution of 3 M TMACI, 0.01 M sodium phosphate (pH 6.8), 1 mM EDTA (pH 7.6), 0.5 % SDS at 1 - 1.5 °C below the T_m; moderate hybridization is effected by a hybridization solution of 6 x SSC and 0.1 % SDS or 3 M TMACI, 0.01 M sodium phosphate (pH 6.8), 1 mM EDTA (pH 7.6), 0.5 % SDS, 100 µg/ml denatured salmon sperm DNA and 0.1 % nonfat dried milk, hybridization temperature of 2 - 2.5 °C below the

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T_m, final wash solution of 3 M TMACI, 0.01 M sodium phosphate (pH 6.8), 1 mM EDTA (pH 7.6), 0.5 % SDS at 1 - 1.5 °C below the T_m, final wash solution of 6 x SSC, and final wash at 22 °C; whereas mild hybridization is effected by a hybridization solution of a hybridization

5   solution of 6 x SSC and 1 % SDS or 3 M TMACI, 0.01 M sodium phosphate (pH 6.8), 1 mM EDTA (pH 7.6), 0.5 % SDS, 100 μg/ml denatured salmon sperm DNA and 0.1 % nonfat dried milk, hybridization temperature of 37 °C, final wash solution of 6 x SSC and final wash at 22 °C.

10   According to an additional aspect of the present invention there is provided a single stranded polynucleotide of at least 18 contiguous nucleotides of SEQ ID NO:27.

According to yet an additional aspect of the present invention there is provided a genetically modified cell expressing the polynucleotide

15   described herein in a sense or antisense orientation.

The present invention successfully addresses the shortcomings of the presently known configurations by providing avian leptin-receptor sequences which are shown herein to share little homology with corresponding mammalian genes and which may therefore be employed to

20   use such cloned genes to control the appetite and other aspects of energy balance in avians, commercially bred poultry in particular. Additional objects and advantages of the present inventions will become apparent reading the description of the preferred embodiments that follows.

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BRIEF DESCRIPTION OF THE DRAWINGS

The invention herein described, by way of example only, with reference to the accompanying drawings, wherein:

5   FIGs. 1a-b show steps of a Southern blot analysis of chicken leptin-receptor phage 1. 1a - restriction enzyme digestion and separation on 1 % agarose gel stained with ethidium bromide. 1b - blotting and hybridization with a human leptin-receptor probe. Washings were with 0.5 x SSC/0.1 % SDS at 42 °C. Exposure was for 3 days. The 1.4 kb Eco RI-Pvu II and the 7

10   kb Eco RI fragments were subcloned and further analyzed.

FIG. 2 shows the nucleotide sequence of the Eco RI-PVU II fragment derived from phage 1 and a comparison thereof to human leptin-receptor exons 9 and 10. This fragment was subcloned in Blue-Script (BS) and sequenced with primers from the BS cloning polylinker. The upper

15   sequence is the chicken leptin-receptor sequence, whereas the lower sequence is the human leptin-receptor exons 9 and 10 sequence. Exonal sequences are shown in bold face.

FIG. 3 shows RT-PCR amplification of chicken leptin-receptor cDNA fragments. Samples of mRNA were prepared from female Leghorn

20   chicken hypothalamus and fat. First-strand cDNA was prepared using random hexamer primers. Exon 9 (forward) and exon 15 (reverse) derived primers served for PCR amplification. The resulting PCR products were electrophoresed on a 1 % agarose gel, blotted onto a nitrocellulose filter, hybridized with a chicken derived exons 9 and 10 leptin-receptor probe,

25   washed and autoradiographed for 4 hours with Kodak BioMax MR X-ray film at -70 °C.

FIGs. 4a-b show steps of a Northern blot analysis of chicken leptin-receptor in the hypothalamus. 4a - Hybridization was performed with a chicken derived leptin-receptor probe under stringent wash conditions.

30   Autoradiography was for 4 days with Kodak BioMax MR X-ray film at -70

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°C. 4b - Ethidiume bromide staining of the RNA indicates the integrity of the RNA and provides for ribosomal RNA (28S and 18S) size reference.

FIG. 5 shows PCR amplification results of DNA derived from a chicken adipose tissue derived cDNA library. DNA was extracted from a
5   sample of the cDNA library and served as template for PCR amplifications in which primers designed according to the so far characterized chicken leptin-receptor exons 10 and 15 and the phage polylinker sequence (T7 and T3) were employed. An example of amplification to the 3' end is shown in the Figure. The first PCR was performed with primers from the phage
10 cloning polylinker (either T3 or T7) and with a chicken leptin-receptor exons 10 forward primer (10F), with touchdown cycles from 65 to 55 °C. Nested PCR was then performed with chicken leptin-receptor 15 forward primer (15F). PCR products were electrophoresed on 1 % agarose gel and were stained with ethidium bromide and photographed under UV
15 illumination. A negative image is shown.

FIG. 6 shows amplification of cDNA ends using the Marathon RACE kit from Clontech. First (1st) and second (2nd) amplifications were performed according to the kit's instruction protocol.

FIGs. 7 shows amplification of chicken leptin-receptor sequences
20 from DNA of chicken leptin-receptor genomic phages 1 and 2. Amplification to the 5' end with T7 T3 phage primers and chicken leptin-receptor exon 10 reverse primer is shown for two independent clones (phage 1 and 2).

FIG. 8 is a schematic presentation of the two genomic phages and the
25 approach which eventually led to the identification and isolation of exons 6-20 of the chicken leptin-receptor gene.

FIG. 9a provides the nucleotide sequence of the coding region (exons 3-20) of the chicken leptin-receptor gene according to the present invention.

FIG. 9b provides the translation of exons 3-20 of the chicken leptin-
30 receptor gene.

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FIG. 10 is a schematic presentation four phages shown to include chicken leptin-receptor genomic DNA sequences. The first two (phages 1 and 2) has already been described in Figure 8 above. Phages 3 and 4 were identified in an additional screen of a chicken genomic DNA library.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is of polynucleotide and polynucleotide sequences of avian leptin-receptor which can be used, in a variety of ways, to study, modulate, select for, and control appetite and other aspects associated with energy balance in avians. Specifically, the present invention can be used to provide an assay which will enable screening for leptin agonists and antagonists; to provide for the ability to attenuate leptin activity in vivo, to thereby manipulate food intake, fat deposition, puberty and fertility in poultry; and to isolate an avian leptin gene.

The principles and operation of the present invention may be better understood with reference to the drawings and accompanying descriptions.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not limited in its application to the details of construction and the arrangement of the components set forth in the following description or illustrated in the drawings. The invention is capable of other embodiments or of being practiced or carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting.

While reducing the present invention to practice, the isolation of a chicken leptin-receptor gene was attempted. As further detailed hereinunder, albeit the knowledge derived from mammalian leptin-receptor genes, this task has proven difficult and non-trivial. In retrospect it can be concluded that this was due to a number of reasons, including, but not

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limited to, (i) the length and number of introns spacing the 20 exons of the gene; (ii) the very low representation of chicken leptin-receptor mRNA in chicken tissues; and therefore (iii) the low (or no) representation of cDNA sequences corresponding to chicken leptin-receptor in an adipose

5   tissue derived cDNA library; (iv) the low homology with mammalian leptin-receptor genes; and (v) the difficulty to PCR amplify chicken leptin-receptor derived sequences, which, in most cases required nested amplification and resulted in non-specific products.

Thus, the following path was taken while reducing the present

10 invention to practice. A human leptin-receptor probe corresponding to exons 9-10, which include regions conserved among other mammalian leptin-receptor genes was prepared. This probe served to screen an adipose tissue derived chicken cDNA library, but no positive clones were identified even under the mildest hybridization conditions, although fat tissue is

15 known to express the leptin-receptor in mammals and could be readily detected. The same probe was then used to screen a chicken genomic library and several genomic clones were identified and subsequently isolated and analyzed to reveal chicken leptin-receptor exons 9 and 10. These exons, which are expected to be most conserved, showed an average

20 homology of 75 % to the corresponding human derived sequences. Retrospectively it was found out that this region shows the highest homology between chicken leptin-receptor and corresponding mammalian leptin-receptors.

Since the leptin-receptor gene in mammals is estimated to be about

25 100 kb long, further efforts were directed, at first, at isolating cDNA sequences of the chicken leptin-receptor gene. Thus, a chicken exon 9 and 10 leptin-receptor probe was used to re-screen the cDNA library, yet, again this effort did not result in isolation of positive cDNA clones. Then, the use of a longer probe was attempted. Subcloning and sequencing of a larger

30 fragment from the genomic clone isolate, and comparing the sequence

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thereof to the GeneBank database led to the identification of chicken

leptin-receptor exon 15. Using RT-PCR with a reverse primer from exon

15 and a forward primer from exon 9 yielded a 1 kb cDNA fragment. This

longer fragment was used to screen the cDNA library, yet again this

5   screening effort did not result in isolation of positive cDNA clones. At this

point is was assumed that the cDNA library is poor in chicken leptin-

receptor derived sequences due to low level of expression thereof in chicken

adipose cells. This assumption is reasonable based on the fact that (i) the

cDNA library employed is known to be highly representative; and (ii) that

10   while attempting to RT-PCR amplify chicken leptin-receptor sequences, the

use of nested amplification was always required. Further attempts to use

several rapid amplification of cDNA ends (RACE) procedures and to fish

mRNA sequences using biotinylated oligonucleotides, as further described

in the Examples section that follows, all failed, reassuring the above

15   assumption that the level of expression of leptin-receptor mRNA in chicken

adipose cells is low.

At this point it was decided to take a new approach. The new

approach was to isolate the extreme coding exons of the chicken leptin-

receptor gene and to employ RT-PCR to then bridge over the missing exons.

20   To this end, probes derived from the mouse leptin-receptor gene exons 3

and 20, which are the extreme coding exons, were prepared by RT-PCR and

the genomic library was screened once more using these probes, however,

no positive clones were isolated. Indeed, following the isolation and

sequencing of exon 20 of the chicken leptin-receptor, it was found that the

25   level of homology between the chicken and the mouse sequences is too low

to sustain hybridization.

The bridging concept was then applied to large fragments of DNA

which were amplified by PCR from DNA derived from the genomic isolates

using leptin-receptor primers in combination with primers hybridizing with

30   the library vector (T3 and T7). Due to the expected length of the amplified

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segments, amplification was attempted, at first, employing high-fidelity enzymes for long template PCR, however, no positive PCR products were obtained. Surprisingly, while attempting the same experimental protocol with a conventional enzyme, not at all recommended for long template PCR, specific positive bands were obtained, which enabled the identification of exons 6 and 20 of the chicken leptin-receptor gene. RT-PCR bridging using exon 6 and exon 20 primers enabled to obtain a cDNA spanning exons 6-20 of the chicken leptin-receptor gene, which cDNA was subsequently cloned and sequenced. Encouraged by the success of this approach, additional genomic clones extending to the 5' region of the chicken leptin-receptor gene were isolated–for the characterization of the missing coding exons 3-5. Purified phage DNA was digested with EcoRI enzyme and the resulting fragments were subcloned in pGM-T vector (Promega). Sequencing 15 independently isolated subclones led to the identification of exons 5 and 4. Exon 3 was dificult to identify because of its small size and low sequence similarity. Therefore the RACE procedure was performed again using the Marathon kit (Clontech), this time employing an exon 6 reverse primer (6RII) and an exon 4 reverse nested primer (4R). Template RNA (0.6 µg polyA+) was prepared from 20 White-Leghorn chickens chicken choroid plexi. RACE products were subcloned into the pGM-T vector and sequencing of 5 independently isolated subclones confirmed the sequence of exon 3. Conformation was done in addition by bridging-PCR using exon 3 primer and exon 10 primer. Thereby a full-length chicken leptin-receptor cDNA, exon 3 to 20 was obtained.

Thus, while reducing the present invention to practice, the full length chicken leptin-receptor gene was cloned, analyzed and sequenced. Cloning the chicken leptin-receptor gene represents the first non-mammalian derived isolated leptin-receptor gene. It is shown herein that this newly isolated gene shares about 60 % homology at the nucleotides level with

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corresponding mammalian genes, whereas a much higher level of homology characterizes these mammalian genes among themselves.

According to one aspect of the present invention there is provided an isolated polynucleotide. The isolated polynucleotide according to the present invention includes a nucleic acid sequence at least 70 % identical, preferably, at least 80 % identical, preferably, at least 85 % identical, more preferably, at least 90 % identical, most preferably 90-100 % identical with a nucleic acid encoding a polypeptide as set forth in SEQ ID NO:28 or a portion thereof longer than 30, preferably longer than 50, more preferably longer than 70, more preferably longer than 100, yet more preferably, longer than 150 contiguous amino acids, as determined using the Smith-Waterman algorithm and the following parameters: Matrix = Blosum62, gapopen = 10.0 and gapextend = 0.5.

The above specified degree of sequence identity is expected to include leptin-receptor sequences derived from avians and reptiles which are evolutionary close, yet, and as further shown in Table 2 in the Examples section that follows, mammalian leptin-receptor genes share only about 60 % nucleotide sequence identity with the chicken gene.

According to another aspect of the present invention there is provided an isolated polynucleotide which includes a nucleic acid sequence of at least 18, preferably at least 25, more preferably at least 50 nucleotides long, hybridizable with a nucleic acid encoding a polypeptide as set forth in SEQ ID NO:28 under a hybridization selected from the group consisting of stringent hybridization, moderate hybridization and mild hybridization.

According to another aspect of the present invention there is provided a single stranded polynucleotide of at least 18, preferably at least 25, more preferably at least 30, 40, 50, 60, 70 or at least 80 nucleotides hybridizing with a nucleic acid sequence as set forth in SEQ ID NO:27 under a hybridization selected from the group consisting of stringent hybridization, moderate hybridization and mild hybridization.

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According to the present invention, hybridization for long nucleic acids (above 200 bp in length) is effected by stringent or moderate hybridization, wherein stringent hybridization is effected by a hybridization solution of 6 x SSC, 5 x Denhardt's solution, 1 % SDS and 100 g/ml denatured salmon sperm DNA, hybridization temperature of 65 °C, final wash solution of 0.1 x SSC and final wash at 60 °C, whereas moderate hybridization is effected by a hybridization solution of 6 x SSC, 5 x Denhardt's solution, 1 % SDS and 100 g/ml denatured salmon sperm DNA, hybridization temperature of 58 °C, final wash solution of 0.5 x SSC and final wash at 50 °C.

Further according to the present invention, hybridization for shorter nucleic acids (below 200 bp in length, e.g. 18-40 bp in length) is effected by stringent, moderate or mild hybridization, wherein stringent hybridization is effected by a hybridization solution of 6 x SSC and 1 % SDS or 3 M TMACI, 0.01 M sodium phosphate (pH 6.8), 1 mM EDTA (pH 7.6), 0.5 % SDS, 100 g/ml denatured salmon sperm DNA and 0.1 % nonfat dried milk, hybridization temperature of 1 - 1.5 °C below the T_m, final wash solution of 3 M TMACI, 0.01 M sodium phosphate (pH 6.8), 1 mM EDTA (pH 7.6), 0.5 % SDS at 1 - 1.5 °C below the T_m; moderate hybridization is effected by a hybridization solution of 6 x SSC and 0.1 % SDS or 3 M TMACI, 0.01 M sodium phosphate (pH 6.8), 1 mM EDTA (pH 7.6), 0.5 % SDS, 100 g/ml denatured salmon sperm DNA and 0.1 % nonfat dried milk, hybridization temperature of 2 - 2.5 °C below the T_m, final wash solution of 3 M TMACI, 0.01 M sodium phosphate (pH 6.8), 1 mM EDTA (pH 7.6), 0.5 % SDS at 1 - 1.5 °C below the T_m, final wash solution of 6 x SSC, and final wash at 22 °C; whereas mild hybridization is effected by a hybridization solution of a hybridization solution of 6 x SSC and 1 % SDS or 3 M TMACI, 0.01 M sodium phosphate (pH 6.8), 1 mM EDTA (pH 7.6), 0.5 % SDS, 100 μg/ml denatured salmon sperm DNA and 0.1 % nonfat dried

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milk, hybridization temperature of 37 °C, final wash solution of 6 x SSC
and final wash at 22 °C.

The above specified hybridization conditions, the stringent
hybridization conditions in particular, allow detectable hybridization
between avian and reptile derived leptin-receptor sequences, yet does not
allow hybridization between these sequences and mammalian derived
leptin-receptor sequences.

According to a preferred embodiment of the present invention, the
nucleic acid sequence is as set forth in SEQ ID NO:27, or a portion thereof
longer than 90, 100, 150, 200 or preferably 300 contiguous nucleotides and
natural (e.g., allelic and non-allelic) and man induced variants (e.g.,
mutations and innocuous or substantially innocuous alterations) thereof.

The polypeptide encoded by the polynucleotide of the present
invention is preferably capable of binding leptin under physiological
conditions. In other words, according to preferred embodiments of the
present invention, the polypeptide encoded by the polynucleotide of the
present invention includes a leptin binding site. In mammals the leptin
binding site of the leptin-receptor is encoded by exons 8-13.

According to another preferred embodiment of the present invention
the isolated polynucleotide further includes a vector ligated to the nucleic
acid sequence herein described. Such a vector can be a phage vector, a viral
vector, a plasmid, a cosmid, a phagemid, an artificial chromosome and the
like and is characterized in that it can propagate within cells. The vector is
preferably an expression vector from which sense and antisense sequences
are expressible to thereby up or down regulate the level of leptin-receptor
mRNA levels in avians. In this case, the vector includes expression control
elements, such as, but not limited to, a promoter and one or more enhancers.
Such expression control elements can be tissue specific, so as to direct
tissue specific regulation of leptin-receptor mRNA levels. These vectors
can be used to genetically modify cells to thereby obtain a genetically

modified cell expressing the polynucleotide described herein in a sense or antisense orientation. Alternatively, the vector can be of a type suitable for gene knock-out, gene knock-in or for overexpression in transgenic animals. In this case the vector preferably includes both positive and negative selection markers, as well known in the art. The vector is preferably integrateable into the genome of the host.

The polynucleotide according to the present invention can be a product of a polymerase chain reaction or any other reaction used for amplification of nucleic acid sequences, such as strand displacement amplification and the like. Alternatively, it can be a product derived from a clone by, for example, digestion with restriction endonucleases.

According to yet another aspect of the present invention there is provided a recombinant leptin-receptor protein. The protein according to the present invention includes an amino acid sequence at least 70 %, 75 %, 80 %, 88 %, or preferably at least 90 % or at least 95 % identical with SEQ ID NO:28 or a portion thereof longer than 30, 40, 50, 70, 90, 150, 200, 300, 500, 700 or preferably longer than 1000 contiguous amino acids of SEQ ID NO:28, as determined using the Smith-Waterman algorithm and the following parameters: Matrix = Blosum62, gapopen = 10.0 and gapextend = 0.5.

Thus, the recombinant proteins herein described differ from mammalian leptin-receptor proteins which exhibit only about 50 % identity with SEQ ID NO:28 (see Table 1 in the Examples section that follows).

According to a preferred embodiment of the present invention the amino acid sequence is as set forth in SEQ ID NO:28 and natural and man induced variants thereof.

The protein according to the present invention can be used for the manufacture of anti-avian leptin-receptor antibodies, both poly and monoclonal antibodies by means well known to the skilled artisan.

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A soluble form of the protein according to the present invention can be used as a drug to control leptin activity because administration of such a soluble form to the blood system of avians will result in capture of circulating leptin therefrom. It will be appreciated in this respect that in mammals, one of the alternatively spliced variants of the leptin-receptor, OBR-e, encodes a soluble form of the receptor, which lacks the intracellular and the trans-membrane domains (Fei H *et al.*, 1997; Lollmann *et al.*, 1997). In an *in vitro* system, the soluble receptor blocks binding of leptin to the longer form of the membranal leptin-receptor (Liu *et al.*, 1997), suggesting that the soluble receptor could normally act as an antagonist of leptin activity. The soluble form can be administered as a purified protein or small peptide derivatives thereof, or alternatively it can be introduced by genetic engineering (gene therapy) techniques by providing avians (e.g., transgenic avians) including cells which have been genetically modified to produce and secrete the soluble form of the receptor. In both cases possible effects could involve food intake, thermoregulation, fat deposition, puberty, fertility, immunity, angiogenesis and the like.

In addition, leptin-receptor cDNA according to the present invention can be used to establish a bioassay for chicken leptin activity in cultured cells. Ba/F3 cells are murine hematopoietic cells which dependent on IL-3 for survival and proliferation. However, expression of the long form of the leptin-receptor therein confers Ba/F3 cells IL-3 independent, provided that the growing cells are subjected to leptin, e.g., human leptin (see, for example, Gainsford *et al.*, 1996). Such genetically modified cells can be used in an assay for monitoring mammalian leptin levels in biological samples. This assay system is expected to be much less or not responsive at al to avian leptins. Expressing the polynucleotide according to the present invention in the Ba/F3 cell line is expected to render these cells IL-3 independent in the presence of avian leptin. Such a genetically modified cell line can therefore be used to evaluate biological activity of chicken

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leptin and to screen libraries of potential leptin agonists and antagonists. It will be appreciated that other cells lines or yeast cells which depend on serum factors which bind cellular receptors which interact with Jak kinase (JAK), and the signal transducer and activator of transcription (STAT) proteins for signal transduction can replace the Ba/F3 cell line to otherwise provide a similar assay system for monitoring avian leptin levels, agonists and antagonists thereof. This assay can be used to screen avian live-stocks for individuals exhibiting exceptionally high or exceptionally low blood leptin levels. Some of these individuals are expected to include leptin or leptin-receptor gene mutations leading to either up or down regulation. Such mutant avians can be employed for selected breeding and the mutated genes thereof can be isolated.

The polynucleotide and recombinant protein according to the present invention can provide a tool for cloning avian leptin genes and/or isolating avian leptin proteins by means of, for example, affinity chromatography, yeast two hybrid system, or screening of expression libraries with labeled receptor protein. It will be appreciated in this respect that the effect of leptin or leptin agonists on the onset of puberty, could significantly increase the profitability in the egg industry and breeder flocks.

It is expected that intervention with the endogenous activity of avian leptin using an avian leptin-receptor will provide a replacement for the forced molting procedure which is employed to improve performance and profitability of old laying hens (Rolon *et al.*, 1993; Hurwitz *et al.*, 1995). The forced molting procedure is typically effected by a short period of feed withdrawal, which results in cessation of production, involution of the reproductive tract, and loss of primary feathers (as reviewed by Brake, 1993).

Additional objects, advantages, and novel features of the present invention will become apparent to one ordinarily skilled in the art upon examination of the following examples, which are not intended to be

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limiting.   Additionally, each of the various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below finds experimental support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions, illustrate the invention in a non limiting fashion.

Generally, the nomenclature used herein and the laboratory procedures in recombinant DNA technology described below are those well known and commonly employed in the art.   Standard techniques are used for cloning, DNA and RNA isolation, amplification and purification. Generally enzymatic reactions involving DNA ligase, DNA polymerase, restriction endonucleases and the like are performed according to the manufacturers' specifications.   These techniques and various other techniques are generally performed according to Sambrook *et al.*, 1989, which is incorporated by reference as if fully set forth herein. Other general references are provided throughout this document.   The procedures therein are believed to be well known in the art and are provided for the convenience of the reader.   All the information contained therein is incorporated herein by reference.

In an attempt to elucidate whether avians posses a mechanism for regulating appetite and other aspects of energy balance, such as, but not limited to, food consumption, energy expenditure, puberty, fertility and immunity, which is similar to that found in mammals, and while reducing the present invention to practice, the cloning of a chicken leptin-receptor has been exercised.

Preparing human leptin-receptor probe for screening libraries: To clone a chicken leptin-receptor homolog gene, a probe was generated according to a sequence corresponding to conserved regions of mammalian leptin-receptor genes, which are expected to encode active domains, and as

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such are likely to be more preserved across the animal kingdom, including in avians.

According to these guidelines, a fragment of about 1 kb which includes exons 6 to 13 of the human leptin-receptor gene was generated by reverse transcriptase - polymerase chain reaction (RT-PCR) from human abdominal fat mRNA using human exon 6 forward (13F) 5'-GTCAGTTCAGCCCATAAATATGG-3' (SEQ ID NO:1) and human exon 13 reverse (13R) 5'-TTGCATCATAAACCTCATACAT-3' (SEQ ID NO:2) primers. Following purification, the resulting RT-PCR fragment of the expected size, 1095 bp, was radioactively labeled with ^{32}P-CTP using the random priming kit from Boehringer Mannheim. Specific activity was approximately 10^8 cpm/µg DNA.

Screening a chicken adipose tissue cDNA library with a human leptin-receptor derived probe; extensive screening did not yield positive clones: A commercially available and highly representative lambda ZAP cDNA library prepared from adipose tissue of a seven week old broiler male (Staratagene, Cat. No. 935405) was plated on 80 mm plates, 8 X 10^5 plaques were blotted onto nitrocellulose filters using standard protocols (Sambrook *et al*, 1989) and subsequently screened using the human derived leptin-receptor probe described hereinabove. Several plaques, which appeared to be positive, were identified and isolated, however, secondary screening of these isolated plaques indicated that the signals obtained were false positive signals. Assuming that low level of sequence similarity might be the problem, the screen was repeated using improved and supposedly more suitable hybridization conditions. The membranes were pre-hybridized for two hours at 42 °C in 5 x SSC; 50 % formamide; 5 x Denhardt's solution; 10 % dextran sulfate; 0.1 % SDS; and 100 µg/ml denatured salmon sperm DNA. Hybridization was carried out overnight at 42 °C in the same solution but with addition of the above described ^{32}P-

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labeled   human   derived   probe.   Hybridization   under   these improved conditions, also resulted only in false positive signals.

Screening a chicken genomic DNA library led to the isolation of a chicken leptin genomic fragment: A commercial chicken lambda FIXII genomic library from male white Leghorn chicken (Stratagene, Cat. No. 946401) was then screened with the human leptin-receptor probe described above. 500,000 plaques, roughly representing 5 genome equivalence of the chicken genome (estimating the chicken genome to be roughly 1×10^9 bp) were screened using the improved hybridization conditions detailed hereinabove. Three independent clones were identified and isolated.

As shown in Figures 1a, DNA extracted from one of the clones thus isolated (which is referred to herein as phage 1) was digested with several restriction endonucleases (Eco RI, Pvu II and Hind III) and combinations thereof. The restriction fragments were then size separated using gel-electrophoresis. As shown in Figure 1b, blotting the size separated restriction fragments onto a nitrocellulose filter, and subsequent hybridization thereof with the human leptin-receptor probe described hereinabove, yielded several specific bands in each of the lanes, which hybridized with the human derived probe.

A Pvu II-Eco RI fragment of 1340 bp (Figures 1a-b, SEQ ID NO:3) was then subcloned into a Blue-Script plasmid (Stratagene) and sequenced from the T3 and the T7 ends of the plasmid's cloning polylinker. Computerized comparison of the sequence with GeneBank sequences revealed sequence similarity to mammalian leptin-receptor exons 9 and 10 (SEQ ID NOs:4-5, respectively).

As shown in Figure 2, alignment of the newly isolated sequence with exons 9 and 10 of the human leptin-receptor gene sequence using the Smith-Waterman algorithm (and the following parameters: Matrix = Blosum62, gapopen = 10.0, and gapextend = 0.5) showed a 76 % nucleotide identity to the 291 bp human leptin-receptor exon 9 (SEQ ID NO:4) and a 75 %

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nucleotide identity to the 118 bp human leptin-receptor exon 10 (SEQ ID NO:5). An intron of 160 bp (Figure 2, SEQ ID NO:3) separating the newly identified chicken leptin-receptor exons 9 and 10 showed no sequence similarity to any GeneBank sequence.

Cloning and sequencing the full length cDNA:

Re-screening the chicken cDNA library using a chicken derived probe; extensive screening did not yield positive clones: Since the leptin-receptor gene in mammals is estimated to be about 100 kb long, the option of "chromosome walking" was ruled out at first. Assuming that using a chicken specific probe would increase sensitivity, a preferred approach of re-screening the chicken adipose cDNA library, this time with a chicken leptin-receptor derived probe was adopted.

To this end, mRNA derived from chicken adipose tissue (Leghorn, female) was reverse transcribed using the superscript enzyme (Stratagene) and a random primer (Gibco-BRL), and subsequently PCR amplified using a forward primer derived from chicken exon 9 (9F) - 5'-AAGATACTGACCAGTGTTGGTTC-3' (SEQ ID NO:6); and reverse primer derived from chicken exon 10 (10R) - 5'-ATGCGTTTGGGTTTGCAGACCAT-3' (SEQ ID NO:7). In addition, a pair of nested primers was used for nested PCR amplification - 5'-TGTTTGGTGGCTGAATTTAGCAG-3' (SEQ ID NO:8) and 5'-GTTAAGTACCCATCAGTTTCACAT-3' (SEQ ID NO:9).

Only following amplification with the nested primers, a 230 bp band detectable by ethidium bromide staining was obtained, isolated and verified by DNA sequencing to include a chicken leptin-receptor derived sequence.

Due to the small size of the DNA fragment thus isolated and in order to avoid generation of fragments shorter than 230 bp, labeling was performed using the nested PCR primers and the Klenow fragment (New England BioLabs). The specific activity of the resulting probe was 10^8 cpm/g DNA.

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This probe was used to re- screen the lambda ZAP cDNA library (Staratagene, Cat. No. 935405) as described hereinabove, again yielding only false-positive results.

Generating a longer cDNA probe: In order to achieve a longer
5   cDNA probe of the chicken leptin-receptor gene, a 7 kb Eco RI fragment of the genomic clone described above (see Figure 1) was isolated and subcloned into a Blue-Script vector. This 7 kb fragment was subsequently sequenced from the T7 and T3 ends of the plasmid's cloning polylinker. Computerized comparison of the sequence with GeneBank sequences
10 revealed 63 % sequence identity to human leptin-receptor exon 15.

mRNA derived from chicken adipose tissue (Leghorn, female) was reverse transcribed using the superscript enzyme (Stratagene) and a random primer (Gibco-BRL), and subsequently PCR amplified using the forward primer 9F from chicken exon 9 and a reverse primer derived from chicken
15 exon 15 (15R) - 5'-CACGGCTAGAATTATAATGGTGT-3' (SEQ ID NO:10).

In a subsequent amplification an additional forward nested primer (SEQ ID NO:8) was used in combination with the above reverse primer 15R to yield a 1 kb amplification product which was subsequently isolated and
20 sequenced, and was shown by sequence comparison analysis to include sequences corresponding to exons 9-15 of leptin-receptor genes.

Re-screening the chicken cDNA library using the longer chicken derived probe (exons 9-15); extensive screening did not yield positive clones: The above described 1 kb fragment was randomprime labeled as
25 described hereinabove and served as a probe to re-screen the lambda ZAP cDNA library (Staratagene, Cat. No. 935405) as described hereinabove, again yielding only false-positive results.

Amplification of cDNA from the lambda ZAP cDNA library; extensive effort did not yield positive amplification products: At this point
30 another approach to isolate a longer chicken leptin-receptor cDNA was

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attempted. Employing nested chicken leptin-receptor specific primers and vector specific primers, DNA extracted from the adipose tissue cDNA library (Staratagene, Cat. No. 935405) was PCR amplified in an effort to isolate chicken leptin-receptor sequences. To this end, 0.5 µg of purified

5   library phage DNA, which roughly represents the DNA content of 10^{10} phage particles, which represent several folds of the RNA complexity was employed. DNA was extracted from 100 µl of the original library, and 0.5 µg DNA was used in a PCR reaction with specific chicken leptin-receptor primers (exon 10 reverse (10R) or exon 15 forward (15F)) in combination

10   with primers from the cloning linker of the lambda ZAP-phage (Blue Script cloning polylinker primers BST3 - 5'-CGCCAAGCTCGAAATTAA CCCTCACTA-3' (SEQ ID NO:11) and BST7 - 5'-GCCAGTGAATTGTAATACGACTCACTA-3' (SEQ ID NO:12).

As shown in the negative image of Figure 6, in some cases, bands

15   stainable by ethidium bromide, smaller than 2 kb were detected, yet their purification and subsequent sequencing resulted in unknown sequences.

Rapid amplification of cDNA ends (RACE); extensive effort did not yield positive amplification products: As reported above, screening the lambda ZAP cDNA library (Staratagene, Cat. No. 935405) with several

20   different probes failed to result in positive isolates, suggesting that the chicken leptin-receptor cDNA is either not represented or represented in very low abundance in the library, possibly due to low abundance of leptin-receptor mRNA in chicken adipose tissue.

Cloning a full length chicken leptin-receptor cDNA was then

25   attempted via the rapid amplification of cDNA ends (RACE) approach, using the highly recommended Marathon kit (Clontech).

Chicken abdominal fat and hypothalamic tissue mRNA samples, both tissues are known to highly express leptin-receptor in mammals, were prepared.

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To this end, hypothalamic RNA was extracted from a pool of 30 hypothalamuses of females White-Leghorn chickens. Abdominal fat RNA was extracted from pooled fat derived from two of these females. Both fat and hypothalamus RNA preparations were examined for the presence of leptin-receptor mRNA. As shown in Figure 3, employing chicken leptin-receptor exon 9 forward primer (9F) and exon 15 reverse primer (15R), both RNA preparations directed the formation of low amounts of RT-PCR products, which RT-PCR products were detectable via hybridization with a chicken leptin-receptor probe corresponding to exons 9-10. Furthermore, as shown in Figures 4a-b, the RNA preparation from hypothalamus was subjected to Northern blot analysis using the chicken leptin-receptor probe corresponding to exons 9-15 and yielded two bands of about 5 and 7 kb corresponding in size to the short and long alternatively splices transcripts of the mammalian leptin-receptor genes.

PolyA^{+} RNA molecules were enriched from both these preparations using a mRNA isolation kit (Boehringer Mannheim, Cat. No. 1741985).

For 5' RACE an exon 10 reverse primer (10R) 5'-ATGCGTTTGGGTTTGCAGACCAT-3' (SEQ ID NO:13) and an exon 10 reverse nested primer (10NR) 5'-GTTAAGTACCCATCAGTTTCACAT-3' (SEQ ID NO:14) were employed. For 3' RACE exon 15 forward primer (15F) 5'-GCACCACCTGCTCATTTCCATGGA-3' (SEQ ID NO:15) and exon 15 forward nested primer (15NF) 5'-CACACCATTACAATTCTAGCCGTGA-3' (SEQ ID NO:16) were employed. 5' and 3' RACE reactions were performed following the manufacturer's instructions.

In both 5' and 3' RACE, first amplifications resulted in ethidium bromide stainable smears, whereas second (nested) amplifications yielded faint ethidium bromide stainable bands. Figure 6 shows a representative example.

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Subsequent   amplification,   purification and sequencing of the bands appeared in any of the RACE experiments resulted in either unidentified or non-relevant sequences.

Modifications to the original RACE technique, still did not lead to amplification of chicken leptin-receptor sequences: It is estimated that the difficulty of cloning the chicken leptin-receptor cDNA by RACE was due to its low abundance. Therefore, additional nested primers were designed. For 5' RACE the following primers were employed: exon 15 reverse primer (15R), exon 10 reverse primer (10R) and then exon 10 reverse nested primer (10NR), For 3' RACE the following primers were employed: exon 10 forward primer (10F) - 5'-ATGGTCTGCAAACCCAAACGCAT-3' (SEQ ID NO:17), exon 15 forward primer (15F) and the exon 15 forward nested primer (15NF). This strategy as well resulted only in false bands.

Fishing for chicken leptin-receptor mRNA molecules from a hypothalamic RNA preparation failed to provide chicken leptin-receptor mRNA: Fishing of chicken leptin-receptor mRNA molecules from a hypothalamic RNA preparation was attempted using a chicken leptin-receptor specific oligonucleotide linked to biotin. To this end, hypothalamuses of 30 female Leghorn chickens were pooled, then checked for the expression of chicken leptin-receptor by Northern blot analysis and incubated with a biotinylated chicken leptin-receptor oligonucleotide derived from exon 9 - 5'- Biotin-CTAAATTCAGCCACCAAA-3' (SEQ ID NO:18). Fishing was then performed using streptavidin coated magnetic particles and a magnetic separator (Boehringer Mannheim). A RACE library prepared from this chicken leptin-receptor enriched mRNA failed to lead, however, to amplification of chicken leptin-receptor cDNA sequences.

Screening the genomic library with probes derived from the 5' and the 3' regions of the mouse leptin-receptor gene; extensive screening did not yield positive clones: As so far, the only isolatable chicken leptin-receptor sequences were derived from the chicken genomic library

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described herein. Assuming at this point that the chicken leptin-receptor transcripts are similar to mammalian leptin-receptor transcripts in global exon-intron structure, it was decided to try and isolate genomic clones including exons 3 and 20, which are the first and last translated

5   exons in the longest transcript of the human leptin-receptor gene, and to use the sequence information therefrom to set up an RT-PCR reaction which will provide a cDNA which corresponds to the entire translated sequence of the chicken leptin-receptor gene. To this end, mouse specific leptin-receptor primers: mouse exon 3 forward 5'-

10   TGTGGTTTTGTTACACTGGG-3' (SEQ ID NO:19); mouse exon 4 reverse 5'-TCTTCCCTTCAGTGTTGTCT-3' (SEQ ID NO:20); mouse exon 20 forward 5'- CAAGCATGCAGAATCAGTGA-3' (SEQ ID NO:21); and mouse exon 20 reverse 5'-CAAAGTGTGAGCATCTCTCC-3' (SEQ ID NO:22), were prepared according to the published sequence and were used

15   in an RT-PCR reaction to amplify cDNAs corresponding to exons 3, 4 and 20 of the mouse leptin-receptor gene. Thus, mouse (CD1 strain) abdominal fat total RNA was extracted and was thereafter subjected to RT-PCR. Reverse transcription was effected by the superscript enzyme (Stratagene) and a random primer (Gibco-BRL), followed by 35 PCR cycles: 94 °C, 30

20   seconds, 55 °C, 30 seconds, 72 °C 1 minute, to yield cDNA bands of the expected sizes 310 bp for exons 3 and 4 and 671 bp for exon 20. It will be recalled in this respect that in order to RT-PCR chicken leptin-receptor derived sequences, nested PCR reactions were necessary. The identity of the mouse exons 3 and 20 cDNA probes thus generated was confirmed by

25   DNA sequencing. An initial hybridization was employed to examine whether the genomic DNA clones so far isolated contain the cDNA ends, however, no positive signals were obtained even under the lowest stringency wash conditions. Hybridization was effected in 5 x SSC; 50 % formamide, 5 x Denhardt's solution, 10 % dextran sulfate, 0.5 % SDS and

30   100 g/ml denatured salmon sperm DNA at 42 °C. Final wash was with 1 x

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SSC and 0.1 % SDS at 42 °C. Therefore, the genomic library was re-screened as described above with each of the exon 3 and exon 20 mouse derived probes, however, no positive clones were obtained.

Amplifying chicken leptin-receptor sequences of genomic clones with chicken leptin-receptor primers and vector derived primers: Two PCR amplifications with chicken leptin-receptor specific primers (exon 9 reverse (9R) and exon 15 forward (15F) primers) combined with primers compatible with the phage cloning polylinker, phT7 - 5'-AATACGACTCACTATAGGGCGTCG-3' (SEQ ID NO:23); and phT3 - 5'-CGCGAGCTCAATTAACCCTCACTA-3' (SEQ ID NO:24), respectively, with enzymes recommended for long template PCR amplifications (e.g., Expand long template PCR system and Expand high fidelity PCR system both from Boehringer Mannheim) were independently attempted on DNA extracted from the above described genomic clones (phage 1 and phage 2). However, bands of different sizes were obtained in both reactions and were analyzed to be false-positives.

Surprisingly, and as shown in Figure 7, while using the Super-term DNA polymerase from SR-PRODUCTS, UK, Cat. No. SRP-801 which is not at all recommended for long template PCR as a PCR enzyme, a similar reaction setup resulted in orientation specific chicken leptin-receptor bands. Cycling conditions were 2 minutes at 94 °C, followed by 35 cycles of 94 °C for 30 seconds, 60 °C for 5 seconds and 68 °C for 12 minutes. The dNTP's concentration employed was 0.5 mM. The ethidium-bromide detectable bands were purified using the Wizard PCR purification kit (Promega). Sequencing the 14 kb band derived from phage 1 with the phT7 primer (see Figure 8, phage 1) led to identification of exon 20 of the chicken leptin-receptor gene. This sequence was used to prepare an exon 20 reverse primer (20R) - 5'-AAGCCCACCTGATCTGGAGTTA-3' (SEQ ID NO:25) and in combination with the exon 15 forward primer (15F) described above

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was employed in an RT-PCR to obtain a cDNA covering exons 15-20. This cDNA was subsequently sequenced.

Using the same experimental procedure, exon 6 was identified on another phage previously isolate (Figure 8, phage 2) and led to the
5 completion of the sequence from exon 6 to exon 20 using RT-PCR as described, employing this time the above exon 20 reverse primer (20R) in combination with an exon 6 forward primer (6F) - 5'-ACTGGGAGATACATCTACCAGCA-3' (SEQ ID NO:26).

Completing cloning and sequencing of the full length chicken
10 ***leptin-receptor cDNA:*** Thus, the isolation of exons 6-20 of the chicken leptin-receptor cDNA was effected by identifying sequences derived from extreme exons present on genomic clones, followed by RT-PCR bridging. The chicken leptin-receptor gene structure shares low homology with mammalian corresponding genes, nevertheless, the Exon-intron structure
15 thereof is preserved. It is therefore assumed that 3 additional exons (3-5) are necessary for completing the entire coding sequence, which exons are expected to include the start signal for translation and to have a total length of about 500 bp.

To this end, a probe containing sequences derived from chicken
20 leptin-receptor exon 6 and intron 5 was prepared using DNA of phage 2 (Figure 8) as a template in a PCR reaction effected via the phT7 and an exon 6 reverse primers (6R) - 5'-ACATCAAGGGTGACCAAA-3' (SEQ ID NO:29). This probe was used to re-screen the genomic library. The screening resulted in the identification and isolation of two positive clones
25 (Figure 10, clones 3 and 4).

These clones were further characterized as described hereinbelow. Phage 4 hybridized with a probe derived from exon 6, yet failed to hybridize with a probe derived from exons 9 to 10. Clone 3, on the other hand, hybridized with both of these probes. Direct sequencing of clone 4 purified

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DNA with the phT3 primer indicated that clone 4 includes sequences of exon 6 and sequences present upstream of exon 6.

Phage 4 failed to hybridize with a probe corresponding to mouse exons 3 and 4. This is due to a low sequence homology between the mouse and chicken sequences as was confirmed by partial sequence analysis of phage 4 DNA which indicated that this phage includes at least exons 4 and 5, and most likely, based on the overall size thereof, also exon 3 of the chicken leptin receptor gene.

Sequencing fragments derived from phage 4 (Figure 8) led to the identification of exons 5 and 4. RACE procedure with exon 6 reverse primer (6FII) 5'-CACATGGCACTCACATTTGCCATA-C-3' (SEQ ID NO:30) and a nested exon 4 forward primer (4R): 5'-CGGCAGCAGAAGGACTCGAAGATG-3' (SEQ ID NO:31) led to identification of exon 3 and the ATG translation initiation codon which was found at the expected position as is compared to corresponding mammalian cDNAs.

Figure 9a shows the nucleotide sequence of exons 3-20 of the chicken leptin-receptor gene (SEQ ID NO:27). The translation of this sequence into amino acids is shown in Figure 9b (SEQ ID NO:28).

Tables 1 and 2 below show sequence homologies shared among exons 6-20 of the leptin-receptor genes of mouse (*Mus musculus*), rat (*Rattus norvegicus*), human (*Homo sapiens*), porcine (*Sus scrofa*), and chicken (*Gallus domesticus*). Please note the low sequence homology shared between the chicken leptin-receptor and the corresponding mammalian genes (59-62 % identical nucleotides; 49-51 % identical amino acids; 75-78 % identical or conserved amino acids), as compared to the much higher homology shared among the mammalian genes (80-92 % identical nucleotides; 74-91 % identical amino acids; 90-96 % identical or conserved (similar) amino acids)

TABLE 1

Comparison of leptin-receptor amino acid sequences, from human, porcine, rat, mouse and chicken

	HS	SS	RN	MM	GD
HS	100%	84% (94%)	75.56% (90%)	75% (90%)	50% (76%)
SS		100%	74% (90%)	75% (90%)	51% (78%)
RN			100%	91% (96%)	50% (75%)
MM				100%	49% (75%)
GD					100%

HS - Homo sapiens; SS - Sus scrofa; RN - Rattus norvegicus; MM - Mus musculus; and GD - Gallus domesticus (exons 6-20).

* In parenthesis % similarity, representing conservative changes in amino acids, as determined, for example, according to the following matrix:

```
      A   R   N   D   C   Q   E   G   H   I   L   K   M   F   P   S   T   W   Y   V|
                                                                                  |
    _____|
  A|  i   -   -   -   +   -   -   +   -   -   -   -   -   -   -   +   +   -   -   +|
  R|  -   i   +   -   -   +   +   -   +   -   -   +   -   -   -   -   -   -   -   -|
  N|  -   +   i   +   -   +   +   +   +   -   -   +   -   -   -   +   +   -   -   -|
  D|  -   -   +   i   -   +   +   -   -   -   -   -   -   -   -   +   -   -   -   -|
  C|  +   -   -   -   i   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -|
  Q|  -   +   +   +   -   i   +   -   +   -   -   +   +   -   -   +   -   -   -   -|
  E|  -   +   +   +   -   +   i   -   +   -   -   +   -   -   -   +   -   -   -   -|
  G|  +   -   +   -   -   -   -   i   -   -   -   -   -   -   -   +   -   -   -   -|
  H|  -   +   +   -   -   +   +   -   i   -   -   -   -   -   -   -   -   -   +   -|
  I|  -   -   -   -   -   -   -   -   -   i   +   -   +   +   -   -   -   -   -   +|
  L|  -   -   -   -   -   -   -   -   -   +   i   -   +   +   -   -   -   -   -   +|
  K|  -   +   +   -   -   +   +   -   -   -   -   i   -   -   -   +   -   -   -   -+|
  M|  -   -   -   -   -   +   -   -   -   +   +   -   i   +   -   -   -   -   -   +|
  F|  -   -   -   -   -   -   -   -   -   +   +   -   +   i   -   -   -   +   +   -|
  P|  -   -   -   -   -   -   -   -   -   -   -   -   -   -   i   -   -   -   -   -|
  S|  +   -   +   +   -   +   +   +   -   -   -   +   -   -   -   i   +   -   -   -|
  T|  +   -   +   -   -   -   -   -   -   -   -   -   -   -   -   +   i   -   -   +|
  W|  -   -   -   -   -   -   -   -   -   -   -   -   -   +   -   -   -   i   +   -|
  Y|  -   -   -   -   -   -   -   -   +   -   -   -   -   +   -   -   -   +   i   -|
  V|  +   -   -   -   -   -   -   -   -   +   +   -   +   -   -   -   +   -   -   i|
    ------------------------------------------------------------------------------
```

```
i = identical aa
- = non similar aa
+ = similar aa
```

TABLE 2

Comparison of leptin-receptor nucleotide sequences, from human, porcine, rat, mouse and chicken

	HS	SS	RN	MM	GD
HS	100%	87%	81%	81%	62%
SS		100%	80%	78%	61%
RN			100%	92%	59%
MM				100%	59%
GD					100%

HS - *Homo sapiens;* SS - *Sus scrofa;* RN - *Rattus norvegicus;* MM - *Mus musculus;* and GD - *Gallus domesticus* (exons 6-20).

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

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References:

1. Ahima, R. S., Dushay, J., Flier, S. N., Prabakaran, D., & Flier, J. S. (1997). Leptin accelerates the onset of puberty in normal female mice. J. Clinical Investigation, 99(3), 391-395.

2. Auwerx and Steels, (1998). Leptin. Lancet, 351, 737-742.

3. Bado, A et al (1998). The stomach is a source of leptin. Nature, 394, 790-793.

4. Bailleul, B., Akerblom, I., & Strosberg, A. D. (1997). The leptin-receptor promoter controls expression of a second distinct protein. Nucleic Acids Research, 25(14), 2752-2758.

5. Campfield, L. A., Smith, F. J., & Burn, P. (1996). The OB protein (leptin) pathway - A link between adipose tissue mass and central neural networks. Hormone and Metabolic Research, 28(12), 619-632.

6. Cao, G. Y., Considine, R. V., & Lynn, R. B. (1997). Leptin-receptors in the adrenal medulla of the rat. American Journal of Physiology Endocrinology and Metabolism, 36(2), E448-E452.

7. Caro, J. F., Kolaczynski, J. W., Nyce, M. R., Ohannesian, J. P., Opentanova, I., Goldman, W. H., Lynn, R. B., Zhang, P. L., Sinha, M. K., & Considine, R. V. (1996). Decreased cerebrospinal-fluid/serum leptin ratio in obesity: A possible mechanism for leptin resistance. Lancet, 348(9021), 159-161.

8. Chehab F. F. et al (1997). Early onset of reproductive function in normal female mice treated with leptin. Science 275, 88-90.

9. Cheung, C. C., Thornton, J. E., Kuijper, J. L., Weigle, D. S., Clifton, D. K., & Steiner, R. A. (1997). Leptin is a metabolic gate for the onset of puberty in the female rat. Endocrinology, 138(2), 855-858.

10. Coleman, D.L., (1978). Obese and diabetes: two mutant genes causing diabetes-obesity syndrom in mice. Daibetologias 14, 141-148.

35.

11. Considine, R.V. *et al.*, (1996) Serum   immunoreactive-leptin concentrations in normal-weigh and obese humans. New England J. of Med, 334, 292-294.

12. Cumin, F., Baum, H. P., deGasparo, M., & Levens, N.   (1997). Removal of endogenous leptin from the circulation by the kidney. International Journal of Obesity, 21(6), 495-504.

13. Emilsson, V., Liu, Y. L., Cawthorne, M.   ., Morton, N. M., & Davenport, M. (1997). Expression of the functional leptin-receptor mRNA in pancreatic islets and direct inhibitory action of leptin on insulin secretion. Diabetes, 46(2), 313-316.

14. Gainsford, T. *et al*, (1996).   Leptin can induce proliferation, differentiation, and functional activation of hemopoietic cells. Proc Natl Acad Sci U S A., 93, 14564-8.

15. Fei H. *et al* (1997).   Anatomic localization of alternatively spliced leptin-receptors (Ob-R) in mouse brain and other tissues. Proc Natl Acad Sci U S A. 94, 7001-7005.

16. Friedman, J.M. and Halaas, J., (1998). Leptin and the regulatof body weight in mammals. Nature, 395, 763-770.

17. Fraley, G.S. and Kuenzel, W.J.,(1993). Precocious puberty in chicken (Gallus domesticus) induced by central injections of neuropeptide Y. Life Sci., 52(20), 1649-1656.

18. Halaas, J. L., Boozer, C., BlairWest, J., Fidahusein, N., Denton, D. A., & Friedman, J. M. (1997). Physiological response to long-term peripheral and central leptin infusion in lean and obese mice. PNAS, 94(16), 8878-8883.

19. Havnes, W. G., etal., (1997). Receptor-mediated regional sympathetic nerve action by leptin. J. Clin. Invest., 100, 270-278.

20. Heiman, M. L., Ahima, R. S., Craft, L. S., Schoner, B., Stephens, T. W., & Flier, J. S. (1997). Leptin inhibition of the hypothalamic-

pituitary-adrenal axis in response to stress. Endocrinology, 138(9), 3859-3863.

21. Hetherington, A. W. and Ranson, S. W. (1942). The spontaneous activity and food intake of rats with hypothalamic lesions. Am. J. Physiol., 136, 609-617.

22. Kamohara, S., Burcelin, R., Halaas, J. L., Friedman, J. M., & Charron, M. J. (1997). Acute stimulation of glucose metabolism in mice by leptin treatment. Nature, 389(6649), 374-377.

23. Campfield, L. A., Smith, F. J., & Burn, P. (1996). The OB protein (leptin) pathway - A link between adipose tissue mass and central neural networks. *Hormone and Metabolic Research, 28 (*12), 619-632.

24. Kuenzel W.J., (1994). Central neuroanatomical systems involved in the regulation of food intake in birds and mammals. J Nutrition, 124(8 Suppl):1355S-1370S.

25. Kuenzel, W.J., Douglass, L.W., Davison, B.A.,(1987). Robust feeding following central administration of neuropeptide Y or peptide YY in chicks, *Gallus domesticus*. Peptides, 8(5), 823-828.

26. Kuenzel, W.J. and McMurtry, J. (1988). Neuropeptide Y: brain localization and central effects on plasma insulin levels in chicks. Physiol. Behav. 44, 669-678.

27. Kuenzel, W.J. Snapir, M. and Rexroad, C. E., Jr. (1987). Food intake and biogenic amine concentrations in brain regions off chicks following intracerebroventricular injection of 6-hydroxydopamine. Pharmacol. Biochem. Behav. 27, 257-263.

28. Lee, G. H., Li, C., Montez, J., Halaas, J., Darvishzadeh, J., & Friedman, J. M. (1997). Leptin-receptor mutations in 129 db(3J)/db(3J) mice and NIH fa(cp)/fa(cp) rats. Mammalian Genome, 8(6), 445-447.

29. Leveille, G.A., Romsos, D.R., Yeh Y-Y and O'Hea, E.K. (1975). Poultry Science, 54, 1075-1093.

37

30. Lipkovsky, S.and Yasuda, M. (1966). Hypothalamic lesions, growth and body composition of male chicken. Poultry Science 45, 582-588.

31. Liu, C. L., Liu, X. J., Barry, G., Ling, N., Maki, R. A., & DeSouza, E. B. (1997). Expression and characterization of a putative high affinity human soluble leptin-receptor. Endocrinology, 138(8), 3548-3554.

32. Lollmann, B., Gruninger, S., StrickerKrongrad, A., & Chiesi, M. (1997). Detection and quantification of the leptin-receptor splice variants Ob-Ra, b, and, e in different mouse tissues. Biochemical and Biophysical Research Communications, 238(2), 648-652.

33. Lord, G. M., Matarese G, Howard JK, Baker RJ, Bloom SR, Lechler RI., (1998). Leptin modulates the T-cell immune response and reverses starvation induced immunosuppression. Nature, 394, 897-901.

34. Luoh SM, Di Marco F, Levin N, Armanini M, Xie MH, Nelson C, Bennett GL,Williams M, Spencer SA, Gurney A, de Sauvage FJ. (1997). Cloning and characterization of a human leptin-receptor using a biologically active leptin immunoadhesin. J Mol Endocrinol, 18(1):77-85.

35. Madej, T., Boguski, M. S., & Bryant, S. H. (1995). Threading analysis suggests that the obese gene product may be a helical cytokine. FEBS Letters, 373(1), 13-18.

36. Maffei, M. (1995). Leptin levels in human and rodent: Measurment of plasma leptin and ob

37. mRNA in obese and weight-reduced subjects. Nature Medicin, 1, 1155-1161.

38. Merce J. G. et al (1996). Localization of leptin-receptor mRNA and the long form splice variant (Ob-Rb) in mouse hypothalamus and adjacent brain regions by in-situ hybridization. FEBS Let. 378, 113-116.

38

39. Masuzaki, H *et al* (1997). Nonadipose tissue production of leptin: leptin as a novel placenta-derived hormone in humans. Nature Med, 3, 1029-1033.

40. Murphy J.E. *et al.*, (1997) Long-term correction of obesity and diabetes in genetically obese mice by a single intramuscular injection of recombinant adeno-associated virus encoding mouse leptin. PNAS, 94, 13921-13926.

41. Naggert, J.K., Fricker, L.D., valamov, O., Nishina, P.M., Rouille, Y., Steiner, D.F., Carroll, R.J., Paigen, b.J., and Leiter, E. [H.sapiens], (1995). Hyperinsulinemia in obese fat/fat mice associated with a carboxypeptidase E mutation which reduces enzyme activity. Nature Genet,. 10, 135-142.

42. Richardson, R.D., Boswell, T., Raffety, B.D., Seeley, R.J., Wingfield, J.C.,and Woods, S.C., (1995). NPY increases food intake in white-crowned sparrows: effect in short and long photoperiods. Am J Physiol, 268, R1418-R1422.

43. Schwartz, M. W., Peskind, E., Raskind, M., Boyko, E. J., & Porte, D. (1996a). Cerebrospinal fluid leptin levels: Relationship to plasma levels and to adiposity in humans. Nature Medicine, 2(5), 589-593.

44. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989). "Molecular cloning: a laboratory manual" 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

45. Schwartz, M. W., Seeley, R. J., Campfield, L. A., Burn, P., & Baskin, D. G. (1996b). Identification of targets of leptin action in rat hypothalamus. Journal of Clinical Investigation, 98(5), 1101-1106.

46. Seeley, R. J., vanDijk, G., Campfield, L. A., Smith, F. J., Burn, P., Nelligan, J. A., Bell,S. M., Baskin, D. G., Woods, S. C., & Schwartz, M. W. (1996). Intraventricular leptin reduces food intake and body weight of lean rats but not obese Zucker rats. Hormone and Metabolic Research, 28(12), 664-668.

39

47. Sierra-Honigmann MR, Nath AK, Murakami C, Garcia-Cardena G, Papapetropoulos A, Sessa WC, Madge LA, Schechner JS, Schwabb MB, Polverini PJ, Flores-Riveros JR (1998). Biological action of leptin as an angiogenic factor. Science, 281,1683-1686.

48. Snapir, N., Ravona, [H.sapiens], and Perek, M., (1973). Effect of electrolytic lesions in various regions of the basal hypothalamus in White Leghorn coockerels upon food intake, obesity, blood triglycerides and protein. Poult. Sci., 52, 629-636.

49. Spicer, L. J., & Francisco, C. C. (1997). The adipose obese gene product, leptin: Evidence of a direct inhibitory role in ovarian function. Endocrinology, 138(8), 3374-3379.

50. Taouis, M., Chen, J-W., Daviaud, C., Dupont, J., Derouet & Simon, J. (1998). Cloning the chicken leptin gene. Gene, 208, 239-242.

51. Tartaglia LA, Dembski M, Weng X, Deng N, Culpepper J, Devos R, Richards GJ, Campfield, LA, Clark FT, Deeds J, *et al* (1995). Identification and expression cloning of a leptin-receptor, OB-R. Cell, 29(83), 1263-1271.

52. Tartaglia, L. A. (1997). The leptin-receptor. Journal of Biological Chemistry, 272(10), 6093-6096.

53. Verploegen, S. A. B. W., Plaetinck, G., Devos, R., VanderHeyden, J., & Guisez, Y. (1997). A human leptin mutant induces weight gain in normal mice. FEBS Letters, 405(2), 237-240.

54. Zhang, Y., Proenca, R., Maffel, M., M., B., Leopold, L., & Friedman, J. M. (1994). Positional cloning of the mouse obese gene and its human homologue. Nature, 372, 425-432.

55. Zhang, F. *et al*., (1997). Crystal structure of the obese protein leptin-E100. Nature, 387, -209.

WHAT IS CLAIMED IS:

1.   An isolated polynucleotide comprising a nucleic acid sequence at least 70 % identical with a nucleic acid encoding a polypeptide as set forth in SEQ ID NO:28 or a portion thereof longer than 30 contiguous amino acids, as determined using the Smith-Waterman algorithm and the following parameters: Matrix = Blosum62, gapopen = 10.0, and gapextend = 0.5.

2.   The isolated polynucleotide of claim 1, wherein said nucleic acid sequence is as set forth in SEQ ID NO:27 or a portion thereof longer than 90 contiguous nucleotides and natural and man induced variants thereof.

3.   The isolated polynucleotide of claim 1, wherein said nucleic acid sequence is of an avian.

4.   The isolated polynucleotide of claim 1, wherein said polypeptide is capable of binding leptin under physiological conditions.

5.   The isolated polynucleotide of claim 1, wherein said nucleic acid sequence is as set forth in SEQ ID NO:27.

6.   The isolated polynucleotide of claim 1, further comprising a vector ligated to said nucleic acid sequence.

7.   The isolated polynucleotide of claim 1, wherein said nucleic acid sequence is a product of a polymerase chain reaction.

8.   A genetically modified cell expressing the polynucleotide of claim 1 in a sense or antisense orientation.

9.   An isolated polynucleotide comprising a nucleic acid sequence hybridizable with a nucleic acid encoding a polypeptide as set forth in SEQ ID NO:28 under a hybridization selected from the group consisting of stringent hybridization and moderate hybridization, wherein stringent hybridization is effected by a hybridization solution of 6 x SSC, 5 x Denhardt's solution, 1 % SDS and 100 g/ml denatured salmon sperm DNA, hybridization temperature of 65 °C, final wash solution of 0.1 x SSC and final wash at 60 °C, whereas

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moderate hybridization is effected by a hybridization solution of 6 x SSC, 5 x Denhardt's solution, 1 % SDS and 100 g/ml denatured salmon sperm DNA, hybridization temperature of 58 °C, final wash solution of 0.5 x SSC and final wash at 50 °C.

10.   The isolated polynucleotide of claim 9, wherein said nucleic acid sequence is as set forth in SEQ ID NO:27 or a portion thereof longer than 90 contiguous nucleotides and natural and man induced variants thereof.

11.   The isolated polynucleotide of claim 9, wherein said nucleic acid sequence is of an avian.

12.   The isolated polynucleotide of claim 9, wherein said polypeptide is capable of binding leptin under physiological conditions.

13.   The isolated polynucleotide of claim 9, wherein said nucleic acid sequence is as set forth in SEQ ID NO:27.

14.   The isolated polynucleotide of claim 9, further comprising a vector ligated to said nucleic acid sequence.

15.   The isolated polynucleotide of claim 9, wherein said nucleic acid sequence is a product of a polymerase chain reaction.

16.   A genetically modified cell expressing the polynucleotide of claim 11 in a sense or antisense orientation.

17.   A recombinant protein comprising an amino acid sequence at least 70 % homologous with SEQ ID NO:28 or a portion thereof longer than 30 contiguous amino acids, as determined using the Smith-Waterman algorithm and the following parameters: Matrix = Blosum62, gapopen = 10.0, and gapextend = 0.5.

18.   The recombinant protein of claim 17, wherein said amino acid sequence is as set forth in SEQ ID NO:28 and natural and man induced variants thereof.

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19.   The recombinant protein of claim 17, wherein said amino acid sequence is of an avian.

20.   The recombinant protein of claim 17, wherein said polypeptide is capable of binding leptin under physiological conditions.

21.   The recombinant protein of claim 17, wherein said amino acid sequence is as set forth in SEQ ID NO:28.

22.   A single stranded polynucleotide of at least 18 nucleotides hybridizing with a nucleic acid sequence as set forth in SEQ ID NO:27 under a hybridization selected from the group consisting of stringent hybridization, moderate hybridization and mild hybridization, wherein stringent hybridization is effected by a hybridization solution of 6 x SSC and 1 % SDS or 3 M TMACI, 0.01 M sodium phosphate (pH 6.8), 1 mM EDTA (pH 7.6), 0.5 % SDS, 100 g/ml denatured salmon sperm DNA and 0.1 % nonfat dried milk, hybridization temperature of 1 - 1.5 °C below the T_m, final wash solution of 3 M TMACI, 0.01 M sodium phosphate (pH 6.8), 1 mM EDTA (pH 7.6), 0.5 % SDS at 1 - 1.5 °C below the T_m; moderate hybridization is effected by a hybridization solution of 6 x SSC and 0.1 % SDS or 3 M TMACI, 0.01 M sodium phosphate (pH 6.8), 1 mM EDTA (pH 7.6), 0.5 % SDS, 100 g/ml denatured salmon sperm DNA and 0.1 % nonfat dried milk, hybridization temperature of 2 - 2.5 °C below the T_m, final wash solution of 3 M TMACI, 0.01 M sodium phosphate (pH 6.8), 1 mM EDTA (pH 7.6), 0.5 % SDS at 1 - 1.5 °C below the T_m, final wash solution of 6 x SSC, and final wash at 22 °C; whereas mild hybridization is effected by a hybridization solution of a hybridization solution of 6 x SSC and 1 % SDS or 3 M TMACI, 0.01 M sodium phosphate (pH 6.8), 1 mM EDTA (pH 7.6), 0.5 % SDS, 100 g/ml denatured salmon sperm DNA and 0.1 % nonfat dried milk, hybridization temperature of 37 °C, final wash solution of 6 x SSC and final wash at 22 °C.

23.   A genetically modified cell expressing the recombinant protein of claim 17.

24.   A single stranded polynucleotide of at least 18 contiguous nucleotides of SEQ ID NO:27.
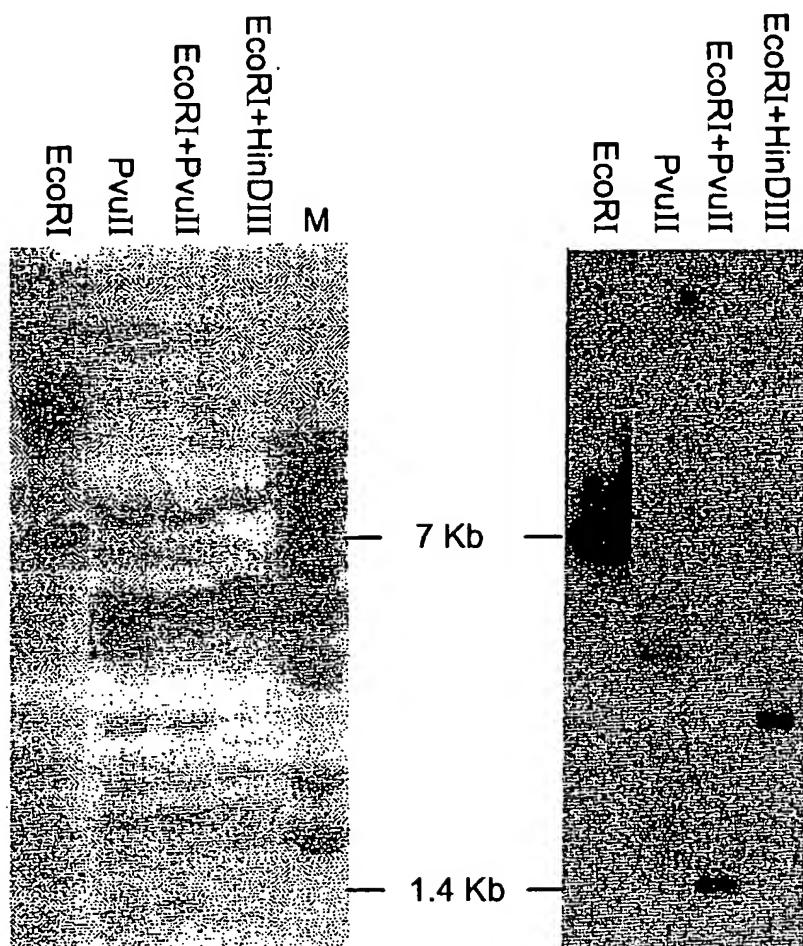
Fig. 1a

Fig. 1b

SEQ ID NO

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   71 ATTAGCATTA AATGATAAAT GCATGGAGAC TGCGCGGTGA TCTCCATGAC ACCCCCATGT CTGGCCCTAT  140

  141 TGCTTTCAGA CCCAGGAGAA GCTGGGCTTT TCACTTATGC TGCCGTGTGT TGTTTTCAAT GTACTGCCCG  210

  211 GGTGTTTTCT GTCAGTTCAT NACCATTTGC CTAGGGACAG TAAAGGTTAT TTTTATTAGA ACTTGTAAAC  280

  281 ATGAGTTATG GAATGAAACA GTCAAGGATC TGTTCCTGCT CCTCTGCCCT ATAGCAATTC GTAATAACAA  350

  351 ACCTGGAAGA ACAGATGATG TAGAGATACT ACGGGATAAT AGGAATTCTC TGTNGCTCTG TAGTTTTTGT  420

  421 TGTTGTTCTG ATTCTGATCT CAGTGGATTT CTTTCTTTTC TTGTAGCTGA AAGTGCTGTA CTTCCCTACT  490
                                                              | ||  | || ||| ||  ||
    1                                     HUMAN EXON 9 ATGTCATATA CTTTCCACCT   20

  491 AAGATACTGA CCAGTGTTGG TTCTAACGTT TCGTTTCATT GCATCTATAA AAACAAAACC CAGAGCGTAG  560
        || || |||| |  | ||||||||  |||||  |||  || ||||||  | |||||||||  ||  ||| |  ||
   21 AAAATTCTGA CAAGTGTTGG GTCTAATGTT TCTTTTCACT GCATCTATAA GAAGGAAAAC AAGATTGTTC   90

  561 CTTCCAAGAA AGATTGTTTG GTGGCTGAAT TTAGCAGAAG AAATCCCAGA AAGTCAGTAT ACGCTTGTGA  630
        | || || |  |||||||||| |||| |||||  |||||  ||     |||| ||  |  ||| |||||||      ||||||
   91 CCTCAAA_AG AGATTGTTTG GTGGATGAAT TTAGCTGAGA AAATTCCTCA AAGCCAGTAT GATGTTGTGA  159

  631 ACGATCGCGT AAGCAAAGTT ACTCTTTTCA ACTTGAAAGC AACCAAACCT AGAGGAAGTT TCTTCTATAA  700
        ||||  ||  |||||||||| |||  ||||||| |  |||| |  |||||||||| |||||| || ||  ||| |
  160 GTGATCATGT TAGCAAAGTT ACTTTTTTCA ATCTGAATGA AACCAAACCT CGAGGAAAGT TTACCTATGA  229

  701 CGCATTGTAC TGTTGCCATC AAAATAGGGA ATGCCATCAT AGATACGCTG AATTATATGT AGTAGGTAAG  770
        ||| |||||  || ||| ||  || ||   ||  |||||||||||  | || |||| ||||||||||   | |
  230 TGCAGTGTAC TGCTGCAATG AACAT__GA ATGCCATCAT CGCTATGCTG AATTATATGT GATTG  291

  771 AGTCCAAATG ATTTTAAATT ACAGTTTCGG TGCATTTTGA TAGTAAGGTC GATCTTCTTA AACTCCACGT  840

  841 AGCTTTTATC AAATAATCAC AGTTTGTAAT GCATGCTTTT TGTGTAAACA AATATATCTT ATTAATAACT  910

  911 TCTAATGTGT TTTTAATTAG ATGTGAATAT CAATATCAAA TGTGAAACTG ATGGGTACTT AACTAAAATG  980
                           |||| |||||| ||||||||  |  |||||||||| |||||||||| ||||||||||
    1                 HUMAN EXON 10  ATGTCAATAT CAATATCTCA TGTGAAACTG ATGGGTACTT AACTAAAATG   50

  981 ACTTGCAGAT GGTCTGCAAA CCCAAACGCA TTGCTCTTGG GGAGTTCCTT GCAGTTAAAA TACCACAGGT 1050
        |||||||||| ||||  | |    ||| |     |  || |||   || _| || ||| || |   || || ||
   51 ACTTGCAGAT GGTCAACCAG TACAATCCAG TCACTTGCGG AAAGCACTTT GCAATTGAGG TATCATAG  118

 1051 GTGTATAGCT TTTAATTCAC GTGTTGTTTG GGAAGTGTTA CTCTGAAAAT ACCATTACAG CAAAGAAACC 1120

 1121 AGGAATAGAT GATAAAATGT GTGAATACAG AATGTGCTCA CTTCCTGTAA GCCAGACTTT TCCTCTGTTG 1190

 1191 GGAACTCCAG AGGTATTGAA CATGTGTGCA AATGAGGGCT GTGGGTATCT GAGTAAGTGA TGTGGATTTT 1260

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 1331 AGTCTAAAAC                                                                   1340
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Fig. 2
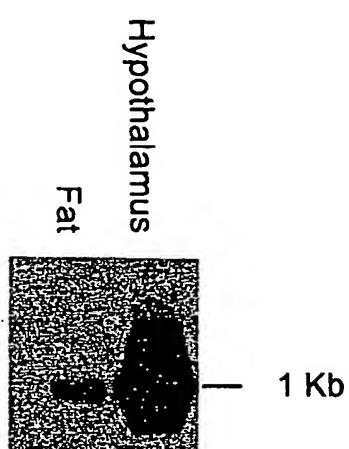
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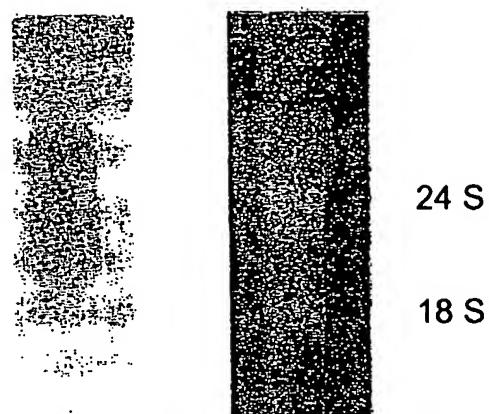
Fig. 4a   Fig. 4b
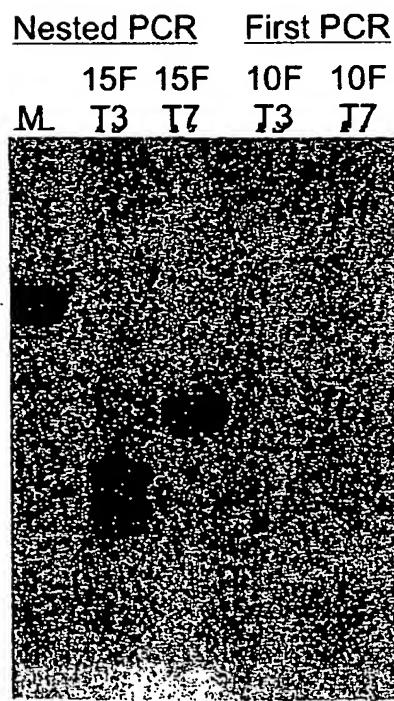
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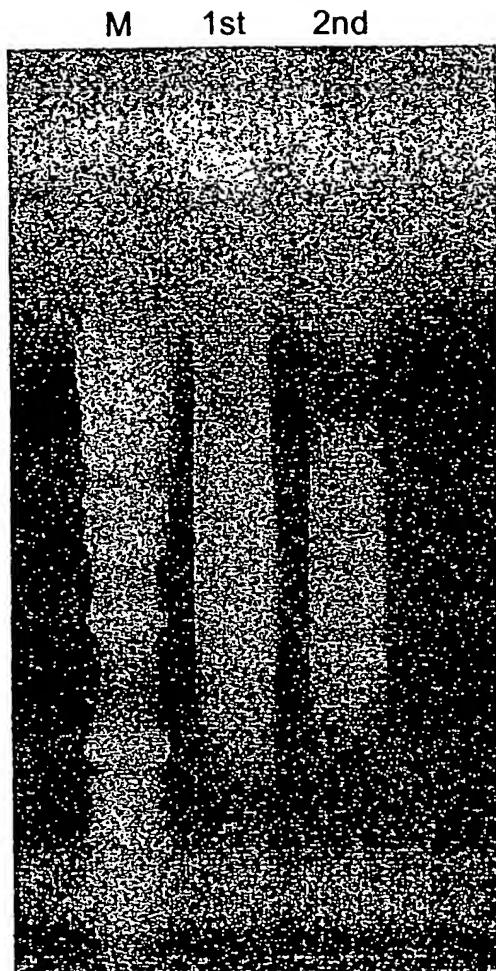
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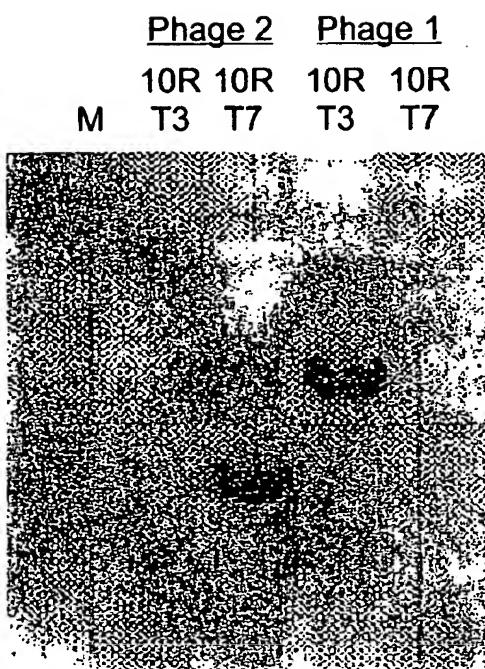
Fig. 7
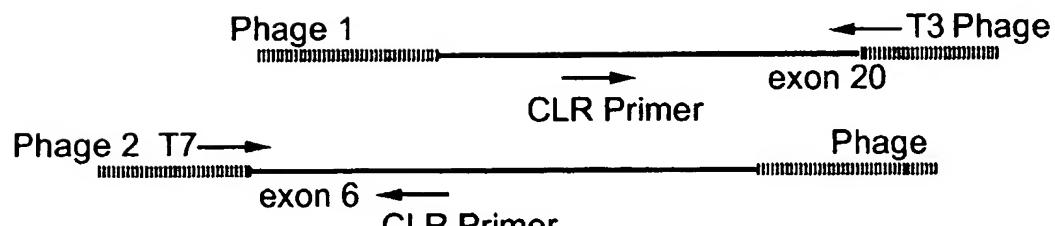
Fig. 8

SEQ ID NO:27

exon 3
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exon 4
GGGCTTTCTTCACGTGGCAGCTGCCCACTGTATGGTCCACGAGATTCATCCCAGGAGCTT
TACATTACCGTGCCTACTGCTGAATGAGACATCTTCGAGTCCTTCTGCTGCCGGAGTTGT
ACGGAGCCGGTCTGGCTGGAGGAGGGAGCATGGAGTTGCTGAAACCAACCCTTCCTTGCT
AATGGATGAGGAGAGTTTTCTGTGTTGCCTTTGGAGCAATAACAACGCCAGTTGTTCTTT
GTACAGTGCAAACATGCAAGCGAGGATGCTTATTCCTTCAGAAATGAGCATCTCTGCATC ·
TCAGGAAAGAG

exon 5
ATTCAAATTGGAACATTGAATGTTGGGTTGAAGGCAAGCTGGATTTGTTAGTTTGTAGCC
TGCAGTTCCCGAAGTTTCACATGAGACTTGATATGAAGGTTCATCTTTTATATGCTGT

exon 6
GTCAGAGCTGTCACTGGGAGATACATCTACCAGCACCCTGAATAGGACTGCCCTGGCTGC
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CCACACTTACGTCATGTGGCTGAAGACTGTGATTGGTCTAACACCTTTTTGGTCACCCTT
GATGTCAGTCAAGCCCATAGACATAG

exon 7
TAAAGCCTGACCTCCCTTTGAATGTGCGTCTAGAGATGACAGAGAGAGGTCAAGTCAAGA
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GGAAATTCAGATCAAATGACTGGCAGGGA

exon 8
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TCCTTTGCTCAAGTGAGGTGCAAGAGTATTTGTGTCCCCGGGTTCTGGAGTGAATGGAGC
ACACTGTATAATCTGAATGGGGAGG

exon 9
TGCTGTACTTCCCTACTAAGATACTGACCAGTGTTGGTTCTAACGTTTCGTTTCATTGCA
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GCCATCAAAATAGGGAATGCCATCATAGATACGCTGAATTATATGTAGTAG

exon 10
ATGTGAATATCAATATCAAATGTGAAACTGATGGGTACTTAACTAAAATGACTTGCAGAT
GGTCTGCAAACCCAAACGCATTGCTCTTGGGGAGTTCCTTGCAGTTAAAATACCACAG

exon 11
CAAAATTTATTGTTCTAACTTTCCAAGTACTCCTCCAGAATCAGAGGTGAAAGAATGCCA
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exon 12
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Fig. 9a

exon 13

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AGCAGATCAGCCTATGCAGCTGTAAAAGATATCCAA

exon 14

GCTCCCTTACATGGCCCTGAGTTTTGGAGAACTGTCACTGAAGATCCAGCAACGGGGCAG
AAGAACGTTACGCTCCTGTGGAAG

exon 15

CCACTGATGAAGAATCACTCACTGTGCAGTGTGAGCCGGTACGTTATAAAGCATCAGACG
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exon 16

GTGAATGCTGTGCAGTCTCTCATTGCTTACCCAGTGAACAGCACGTGTGTGATTTTGACT
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TAT

exon 17

GACCACTTTATTCTGATTGAGAAGTACCGGTTCAGCCTGTACCCCGTGTTTGCTGCAGGA
GTTGGCAAATCCAGAGCCACGGATCAGTTCTCCAAA

exon 18

GATGGGTATGCCAGTCAGACCAGTTCTAACCTCTATATGGTCCTGCCAATAGTTATTTCA
ACCTCCGTGCTGTTGCTTGGAGCGCTGCTGGTTTCGCACCGAAGA

exon 19

ATGAAGAAACTGCTCTGGGAAGATGTTCCGATCCCCAAGAATTGCTCGTGGGCACAAGGT
GTTGATTTTCAGCAG

exon 20

CCTGAAACTTTTGAGCACCTTTTTGTCAAGCACCCTGAAGCAATGTCATTTGAGCCTCTT
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AACTCCAGATCAGGTGGGCTTTACGAACAGAATAAGAATCCCAGATGTCATTTTGATGGA
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TAGAACTCTATGATTCTATGATGGCCCTTCYTGACCTAATTTTCAAATGTGGGCCTTCT
TTTATATAATTTATATAAAAATATATTCAAAGGATGTCTGTTGGCCATGCAGACCCAAGC
ACGCGTCTCCCCATTCAGGAGTAAAGCATCTCTTTATACGTGCTGCCAGACTCTGAGTGA
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TAGGAGTCG

Fig. 9a (continued)

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   1 MetTyrHisGlnIleIleLeuThrMetSerLeuLeuLeuGlyPheLeuHi  17  SEQ ID NO:28

  87 CGTGGCAGCTGCCCACTGTATGGTCCACGAGATTCATCCCAGGAGCTTTA  136
     ||||||||||||||||||||||||||||||||||||||||||||||||||
  17 sValAlaAlaAlaHisCysMetValHisGluIleHisProArgSerPheT  34

 137 CATTACCGTGCCTACTGCTGAATGAGACATCTTCGAGTCCTTCTGCTGCC  186
     ||||||||||||||||||||||||||||||||||||||||||||||||||
  34 hrLeuProCysLeuLeuLeuAsnGluThrSerSerSerProSerAlaAla  50

 187 GGAGTTGTACGGAGCCGGTCTGGCTGGAGGAGGGAGCATGGAGTTGCTGA  236
     ||||||||||||||||||||||||||||||||||||||||||||||||||
  51 GlyValValArgSerArgSerGlyTrpArgArgGluHisGlyValAlaGl  67

 237 AACCAACCCTTCCTTGCTAATGGATGAGGAGAGTTTTCTGTGTTGCCTTT  286
     ||||||||||||||||||||||||||||||||||||||||||||||||||
  67 uThrAsnProSerLeuLeuMetAspGluGluSerPheLeuCysCysLeuT  84

 287 GGAGCAATAACAACGCCAGTTGTTCTTTGTACAGTGCAAACATGCAAGCG  336
     ||||||||||||||||||||||||||||||||||||||||||||||||||
  84 rpSerAsnAsnAsnAlaSerCysSerLeuTyrSerAlaAsnMetGlnAla  100

 337 AGGATGCTTATTCCTTCAGAAATGAGCATCTCTGCATCTCAGGAAAGAGA  386
     ||||||||||||||||||||||||||||||||||||||||||||||||||
 101 ArgMetLeuIleProSerGluMetSerIleSerAlaSerGlnGluArgAs  117

 387 TTCAAATTGGAACATTGAATGTTGGGTTGAAGGCAAGCTGGATTTGTTAG  436
     ||||||||||||||||||||||||||||||||||||||||||||||||||
 117 pSerAsnTrpAsnIleGluCysTrpValGluGlyLysLeuAspLeuLeuV  134

 437 TTTGTAGCCTGCAGTTCCCGAAGTTTCACATGAGACTTGATATGAAGGTT  486
     ||||||||||||||||||||||||||||||||||||||||||||||||||
 134 alCysSerLeuGlnPheProLysPheHisMetArgLeuAspMetLysVal  150

 487 CATCTTTTATATGCTGTGTCAGAGCTGTCACTGGGAGATACATCTACCAG  536
     ||||||||||||||||||||||||||||||||||||||||||||||||||
 151 HisLeuLeuTyrAlaValSerGluLeuSerLeuGlyAspThrSerThrSe  167

 537 CACCCTGAAGAGGACTGCCCTGGCTGCTCAGTGTAACTGCAGTGAGTATG  586
     ||||||||||||||||||||||||||||||||||||||||||||||||||
 167 rThrLeuLysArgThrAlaLeuAlaAlaGlnCysAsnCysSerGluTyrG  184

 587 GCAAATGTGAGTGCCATGTGCCTTCCCCAAGACTCAACCACACTTACGTC  636
     ||||||||||||||||||||||||||||||||||||||||||||||||||
 184 lyLysCysGluCysHisValProSerProArgLeuAsnHisThrTyrVal  200

 637 ATGTGGCTGAAGACTGGGATTGGTCTAACACCTGTTTGGTCACCCTTGAT  686
     ||||||||||||||||||||||||||||||||||||||||||||||||||
 201 MetTrpLeuLysThrGlyIleGlyLeuThrProValTrpSerProLeuMe  217

 687 GTCAGCCAAGCCCATAGACATAGTAAAGCCTGACCTCCCTTTGAATGTGC  736
     ||||||||||||||||||||||||||||||||||||||||||||||||||
 217 tSerAlaLysProIleAspIleValLysProAspLeuProLeuAsnValA  234
```

Fig. 9b

737 GTCTAGAGATGACAGAGAGAGGTCAAGTCAAGATCTGCTGGTCTGAGCCT 786
     ||||||||||||||||||||||||||||||||||||||||||||||||||
234 rgLeuGluMetThrGluArgGlyGlnValLysIleCysTrpSerGluPro 250

787 GTACCGATGCCGTACCCGCTCCGGTGTGAAGTGAACATCTTCTGGAAATT 836
     ||||||||||||||||||||||||||||||||||||||||||||||||||
251 ValProMetProTyrProLeuArgCysGluValAsnIlePheTrpLysPh 267

837 CAGATCAAATGACTGGCAGGGAGTGGTTCAAGTTGCTTTAAATACCTCAT 886
     ||||||||||||||||||||||||||||||||||||||||||||||||||
267 eArgSerAsnAspTrpGlnGlyValValGlnValAlaLeuAsnThrSerL 284

887 TAGACATAGACAATATGCTGCTTGATTCTTCCTCCTTTGCTCAAGTGAGG 936
     ||||||||||||||||||||||||||||||||||||||||||||||||||
284 euAspIleAspAsnMetLeuLeuAspSerSerSerPheAlaGlnValArg 300

937 TGCAAGAGTATTTGTGTCCCCGGGTTCTGGAGTGAATGGAGCACACTGTA 986
     ||||||||||||||||||||||||||||||||||||||||||||||||||
301 CysLysSerIleCysValProGlyPheTrpSerGluTrpSerThrLeuTy 317

987 TAATCTGAATGGGGAGGTGCTGTACTTCCCTACTAAGATACTGACCAGTG 1036
     ||||||||||||||||||||||||||||||||||||||||||||||||||
317 rAsnLeuAsnGlyGluValLeuTyrPheProThrLysIleLeuThrSerV 334

1037 TTGGTTCTAACGTTTCGTTTCATTGCATCTATAAAAACAAAACCCAGAGC 1086
      ||||||||||||||||||||||||||||||||||||||||||||||||||
334   alGlySerAsnValSerPheHisCysIleTyrLysAsnLysThrGlnSer 350

1087 GTAGCTTCCAAGAAGATTGTTTGGTGGCTGAATTTAGCAGAAGAAATCCC 1136
      ||||||||||||||||||||||||||||||||||||||||||||||||||
351   ValAlaSerLysLysIleValTrpTrpLeuAsnLeuAlaGluGluIlePr 367

1137 AGAAAGTCAGTATACGCTTGTGAACGATCGCGTAAGCAAAGTTACTCTTT 1186
      ||||||||||||||||||||||||||||||||||||||||||||||||||
367   oGluSerGlnTyrThrLeuValAsnAspArgValSerLysValThrLeuP 384

1187 TCAACTTGAAAGCAACCAAACCTAGAGGAAGTTTCTTCTATAACGCATTG 1236
      ||||||||||||||||||||||||||||||||||||||||||||||||||
384   heAsnLeuLysAlaThrLysProArgGlySerPhePheTyrAsnAlaLeu 400

1237 TACTGTTGCCATCAAAATAGGGAATGCCATCATAGATACGCTGAATTATA 1286
      ||||||||||||||||||||||||||||||||||||||||||||||||||
401   TyrCysCysHisGlnAsnArgGluCysHisHisArgTyrAlaGluLeuTy 417

1287 TGTAGTAGATGTGAATATCAATATCAAATGTGAAACTGATGGGTACTTAA 1336
      ||||||||||||||||||||||||||||||||||||||||||||||||||
417   rValValAspValAsnIleAsnIleLysCysGluThrAspGlyTyrLeuT 434

1337 CTAAAATGACTTGCAGATGGTCTGCAAACCCAAACGCATTGCTCTTGGGG 1386
      ||||||||||||||||||||||||||||||||||||||||||||||||||
434   hrLysMetThrCysArgTrpSerAlaAsnProAsnAlaLeuLeuLeuGly 450

1387 AGTTCCTTGCAGTTAAAAATACCACAGCAAAATTTATTGTTCTAACTTTCC 1436
      ||||||||||||||||||||||||||||||||||||||||||||||||||
451   SerSerLeuGlnLeuLysTyrHisSerLysIleTyrCysSerAsnPhePr 467

1437 AAGTACTCCTCCAGAATCAGAGGTGAAAGAATGCCATTTCCAGAGGAATC 1486
      ||||||||||||||||||||||||||||||||||||||||||||||||||
467   oSerThrProProGluSerGluValLysGluCysHisPheGlnArgAsnH 484

Fig. 9b (continued)

```
1487 ATTCTTATGAGTGCACATTTCAGCCTGTTTTTCTTTTATCTGGACATACC 1536
     ||||||||||||||||||||||||||||||||||||||||||||||||||
 484 isSerTyrGluCysThrPheGlnProValPheLeuLeuSerGlyHisThr 500
```

```
1537 ATGTGGATTGAGCTTAAGCACTCGCTGGGAACACTTGAATCCTCACCAAC 1586
     ||||||||||||||||||||||||||||||||||||||||||||||||||
 501 MetTrpIleGluLeuLysHisSerLeuGlyThrLeuGluSerSerProTh 517
```

```
1587 TTGTGTCGTTCCAGCAGATGTGGTGAAGCCACTGCCTCCCTCCAACATTA 1636
     ||||||||||||||||||||||||||||||||||||||||||||||||||
 517 rCysValValProAlaAspValValLysProLeuProProSerAsnIleL 534
```

```
1637 AAGCAGAGATCACCAGAAACGATGGGCTGCTGAACGTGAGCTGGACAAAC 1686
     ||||||||||||||||||||||||||||||||||||||||||||||||||
 534 ysAlaGluIleThrArgAsnAspGlyLeuLeuAsnValSerTrpThrAsn 550
```

```
1687 CCCGTGTTTACAAATGATGACCTTAAGTTTCAGATCCGGTGCGCAGTGAA 1736
     ||||||||||||||||||||||||||||||||||||||||||||||||||
 551 ProValPheThrAsnAspAspLeuLysPheGlnIleArgCysAlaValAs 567
```

```
1737 CAGGGAAGAACTCACATGGGAGCTGTATGAAGTTCTAAGCGTACCAACAA 1786
     ||||||||||||||||||||||||||||||||||||||||||||||||||
 567 nArgGluGluLeuThrTrpGluLeuTyrGluValLeuSerValProThrA 584
```

```
1787 GATCAGCTGTGATAGAAGTGCAACTTTGTGTTGAATATATTGTTCAGATC 1836
     ||||||||||||||||||||||||||||||||||||||||||||||||||
 584 rgSerAlaValIleGluValGlnLeuCysValGluTyrIleValGlnIle 600
```

```
1837 CGCTGCAGAGCCCTGGATGGCTTAGGCTACTGGAGCAACTGGAGCAGATC 1886
     ||||||||||||||||||||||||||||||||||||||||||||||||||
 601 ArgCysArgAlaLeuAspGlyLeuGlyTyrTrpSerAsnTrpSerArgSe 617
```

```
1887 AGCCTATGCAGCTGTAAAAGATATCCAAGCTCCCTTACATGGCCCTGAGT 1936
     ||||||||||||||||||||||||||||||||||||||||||||||||||
 617 rAlaTyrAlaAlaValLysAspIleGlnAlaProLeuHisGlyProGluP 634
```

```
1937 TTTGGAGAACTGTCACTGAAGATCCAGCAACGGGGCAGAAGAACGTTACG 1986
     |||||||||||||||||||||||||||||||||||||||||-||||||||
 634 heTrpArgThrValThrGluAspProAlaThrGlyGlnLysAsnValThr 650
```

```
1987 CTCCTGTGGAAGCCACTGATGAAGAATCACTCACTGTGCAGTGTGAGCCG 2036
     ||||||||||||||||||||||||||||||||||||||||||||||||||
 651 LeuLeuTrpLysProLeuMetLysAsnHisSerLeuCysSerValSerAr 667
```

```
2037 GTACGTTATAAAGCATCAGACGTCAGAAAACACCTCGTGGTCAGARTATG 2086
     ||||||||||||||||||||||||||||||||||||||||||||||||||
 667 gTyrValIleLysHisGlnThrSerGluAsnThrSerTrpSerGluTyrV 684
```

```
2087 TCGACAATGGCACCACCTGCTCATTTCCATGGACTGAAAGCACACACACC 2136
     ||||||||||||||||||||||||||||||||||||||||||||||||||
 684 alAspAsnGlyThrThrCysSerPheProTrpThrGluSerThrHisThr 700
```

```
2137 ATTACATTTCTAGCCGTGAATTCAATTGGAGCTTCTTCAGTTAATTTTAA 2186
     ||||||||||||||||||||||||||||||||||||||||||||||||||
 701 IleThrPheLeuAlaValAsnSerIleGlyAlaSerSerValAsnPheAs 717
```

```
2187 TTTAACTCTGTCACAACAAATGAGCACAGTGAATGCTGTGCAGTCTCTCA 2236
     ||||||||||||||||||||||||||||||||||||||||||||||||||
 717 nLeuThrLeuSerGlnGlnMetSerThrValAsnAlaValGlnSerLeuI 734
```

Fig. 9b (continued)

2237 TTGCTTACCCAGTGAACAGCACGTGTGTGATTTTGACTTGGACGCTTTCG 2286
     |||||||||||||||||||||||||||||||||||||||||||||||||
734 leAlaTyrProValAsnSerThrCysValIleLeuThrTrpThrLeuSer 750

2287 CCTCAAATATATGTGATAACATCTTTTATTATTGAGTGGAGAAACCTTAA 2336
     |||||||||||||||||||||||||||||||||||||||||||||||||
751 ProGlnIleTyrValIleThrSerPheIleIleGluTrpArgAsnLeuAs 767

2337 CAAAGAAGAGGAGATGAAGTGGGTGCAAGTTCCTCCAAATATTAGTAAAC 2386
     |||||||||||||||||||||||||||||||||||||||||||||||||
767 nLysGluGluGluMetLysTrpValGlnValProProAsnIleSerLysH 784 ·

2387 ACTATATTTATGACCACTTTATTCTGATTGAGAAGTACCGGTTCAGCCTG 2436
     |||||||||||||||||||||||||||||||||||||||||||||||||
784 isTyrIleTyrAspHisPheIleLeuIleGluLysTyrArgPheSerLeu 800

2437 TACCCCGTGTTTGCTGCAGGAGTTGGCAAATCCAGAGCCACGGATCAGTT 2486
     |||||||||||||||||||||||||||||||||||||||||||||||||
801 TyrProValPheAlaAlaGlyValGlyLysSerArgAlaThrAspGlnPh 817

2487 CTCCAAAGATGGGTATGCCAGTCAGACCAGTTCTAACCTCTATATGGTCC 2536
     |||||||||||||||||||||||||||||||||||||||||||||||||
817 eSerLysAspGlyTyrAlaSerGlnThrSerSerAsnLeuTyrMetValL 834

2537 TGCCAATAGTTATTTCAACCTCCGTGCTGTTGCTTGGAGCGCTGCTGGTT 2586
     |||||||||||||||||||||||||||||||||||||||||||||||||
834 euProIleValIleSerThrSerValLeuLeuLeuGlyAlaLeuLeuVal 850

2587 TCGCACCGAAGAATGAAGAAACTGCTCTGGGAAGATGTTCCGATCCCCAA 2636
     |||||||||||||||||||||||||||||||||||||||||||||||||
851 SerHisArgArgMetLysLysLeuLeuTrpGluAspValProIleProLy 867

2637 GAATTGCTCGTGGGCACAAGGTGTTGATTTTCAGCAGCCTGAAACTTTTG 2686
     |||||||||||||||||||||||||||||||||||||||||||||||||
867 sAsnCysSerTrpAlaGlnGlyValAspPheGlnGlnProGluThrPheG 884

2687 AGCACCTTTTTGTCAAGCACCCTGAAGCAATGTCATTTGAGCCTCTTCTT 2736
     |||||||||||||||||||||||||||||||||||||||||||||||||
884 luHisLeuPheValLysHisProGluAlaMetSerPheGluProLeuLeu 900

2737 CTGGAACCGGAAATAGTGCTGGAAGACATCAGTGTTACTAAAGCTTTGGA 2786
     |||||||||||||||||||||||||||||||||||||||||||||||||
901 LeuGluProGluIleValLeuGluAspIleSerValThrLysAlaLeuGl 917

2787 ACAAGAAGACACGCAAGATTTCTTAGCGCTTGATTCCATGTTCACAAAAC 2836
     |||||||||||||||||||||||||||||||||||||||||||||||||
917 uGlnGluAspThrGlnAspPheLeuAlaLeuAspSerMetPheThrLysP 934

2837 CTGAAGACTCTGAGCACGACTCTGCGTGTCCCAGCAGCCACTTCAGTGGT 2886
     |||||||||||||||||||||||||||||||||||||||||||||||||
934 roGluAspSerGluHisAspSerAlaCysProSerSerHisPheSerGly 950

2887 CGCAGCTCCATGGAGTGCTCCCCCAGTGACCCGACCTCTGGAGAAACAGC 2936
     |||||||||||||||||||||||||||||||||||||||||||||||||
951 ArgSerSerMetGluCysSerProSerAspProThrSerGlyGluThrAl 967

2937 AAGTCAGTCCAACATTAAGTATGCCACTGTTATCAGTAACTCCAGATCAG 2986
     |||||||||||||||||||||||||||||||||||||||||||||||||
967 aSerGlnSerAsnIleLysTyrAlaThrValIleSerAsnSerArgSerG 984

Fig. 9b (continued)

11/11

```
2987 GTGGGCTTTACGAACAGAATAAGAATCCCAGATGTCATTTTGATGGATGT 3036
      ||||||||||||||||||||||||||||||||||||||||||||||||||
 984  lyGlyLeuTyrGluGlnAsnLysAsnProArgCysHisPheAspGlyCys 1000

3037 TTTCTAGCTGAAGACTCCTTGGCTGCAGGTGCTTGCTCAGGTAGCTCCTG 3086
      ||||||||||||||||||||||||||||||||||||||||||||||||||
1001  PheLeuAlaGluAspSerLeuAlaAlaGlyAlaCysSerGlySerSerTr 1017

3087 GGAGCTGGGAAATGAGGCGTTCCTCCTGCTGCCTGACCAGCCTGGCAGCC 3136
      ||||||||||||||||||||||||||||||||||||||||||||||||||
1017  pGluLeuGlyAsnGluAlaPheLeuLeuLeuProAspGlnProGlySerG 1034

3137 AGCCCTGCAAGACCCTCTCCCTTATCTCTTCAGAGGGATTTTCCGAGCCT 3186
      ||||||||||||||||||||||||||||||||||||||||||||||||||
1034  lnProCysLysThrLeuSerLeuIleSerSerGluGlyPheSerGluPro 1050

3187 TCAGATCAGGATGATGCTTTCACAGATGGAGGTAGCCCTGAGCGAGGTCT 3236
      ||||||||||||||||||||||||||||||||||||||||||||||||||
1051  SerAspGlnAspAspAlaPheThrAspGlyGlySerProGluArgGlyLe 1067

3237 CCATTACCTAGGGATAACATCACTGGGCAAAAGAGAAAATGACATTTTTT 3286
      ||||||||||||||||||||||||||||||||||||||||||||||||||
1067  uHisTyrLeuGlyIleThrSerLeuGlyLysArgGluAsnAspIlePheL 1084

3287 TAACAGAAAGTTCCAGACTGATGTGCCATTTCCATACAGCTGATCTGCTC 3336
      ||||||||||||||||||||||||||||||||||||||||||||||||||
1084  euThrGluSerSerArgLeuMetCysHisPheHisThrAlaAspLeuLeu 1100

3337 AGAGGTGTGGGATTTCTTCAGAATACGCCTCCTAATTTAAATGCATTTAT 3386
      ||||||||||||||||||||||||||||||||||||||||||||||||||
1101  ArgGlyValGlyPheLeuGlnAsnThrProProAsnLeuAsnAlaPheIl 1117

3387 CCAGAGTAGCATTAAAGCCATCGTGCCATACGTGCCGCAGTTTCAGATGA 3436
      ||||||||||||||||||||||||||||||||||||||||||||||||||
1117  eGlnSerSerIleLysAlaIleValProTyrValProGlnPheGlnMetT 1134

3437 CTGCTGCCAAGGTGCAAGAGACCACAGAGAACAGCTGT 3474
      ||||||||||||||||||||||||||||||||||||||  -
1134  hrAlaAlaLysValGlnGluThrThrGluAsnSerCys 1146
```

Fig. 9b (continued)

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exon 20

Phage 2   Phage
exon 6   exon 20

Phage 3   Phage
exon 6

Phage 4   Phage   Fig. 10
exon 6
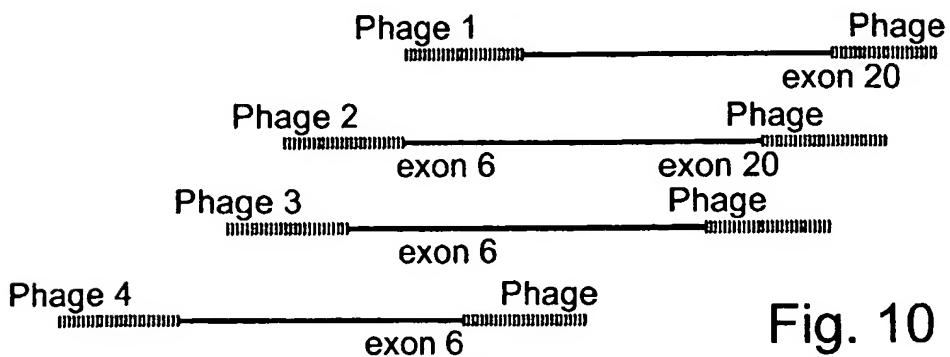
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cttttttcaa tctgaatgaa accaaacctc gaggaaagtt tacctatgac gcattgtact   240

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4

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6

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<400>  22
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<210>  23

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<210>  25

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<212>  DNA

<213>  Synthetic Oligonucleotide



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<210>  26

<211>  23

<212>  DNA

<213>  Synthetic Oligonucleotide



<400>  26
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<210>  27

<211>  3850

<212>  DNA

<213>  Gallus domesticus



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atgtctttgc tgttgggctt tcttcacgtg gcagctgccc actgtatggt ccacgagatt   120

catcccagga gctttacatt accgtgccta ctgctgaatg agacatcttc gagtccttct   180

gctgccggag ttgtacggag ccggtctggc tggaggaggg agcatggagt tgctgaaacc   240

aaccctcctt gctaatggat gaggagagtt ttctgtgttg cctttggagc aataacaacg   300

ccagttgttc tttgtacagt gcaaacatgc aagcgaggat gcttattcct tcagaaatga   360

gcatctctgc atctcaggaa agagattcaa attggaacat tgaatgttgg gttgaaggca   420
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8

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agctggattt gttagtttgt agcctgcagt tcccgaagtt tcacatgaga cttgatatga   480

aggttcatct tttatatgct gtgtcagagc tgtcactggg agatacatct accagcaccc   540

tgaagaggac tgccctggct gctcagtgta actgcagtga gtatggcaaa tgtgagtgcc   600

atgtgccttc cccaagactc aaccacactt acgtcatgtg gctgaagact gggattggtc   660

taacacctgt ttggtcaccc ttgatgtcag ccaagcccat agacatagta aagcctgacc   720

tccctttgaa tgtgcgtcta gagatgacag agagaggtca agtcaagatc tgctggtctg   780

agcctgtacc gatgccgtac ccgctccggt gtgaagtgaa catcttctgg aaattcagat   840

caaatgactg gcagggagtg gttcaagttg ctttaaatac ctcattagac atagacaata   900

tgctgcttga ttcttcctcc tttgctcaag tgaggtgcaa gagtatttgt gtccccgggt   960

tctggagtga atggagcaca ctgtataatc tgaatgggga ggtgctgtac ttccctacta   1020

agatactgac cagtgttggt tctaacgttt cgtttcattg catctataaa aacaaaaccc   1080

agagcgtagc ttccaagaag attgtttggt ggctgaattt agcagaagaa atcccagaaa   1140

gtcagtatac gcttgtgaac gatcgcgtaa gcaaagttac tcttttcaac ttgaaagcaa   1200

ccaaacctag aggaagtttc ttctataacg cattgtactg ttgccatcaa aataggaat   1260

gccatcatag atacgctgaa ttatatgtag tagatgtgaa tatcaatatc aaatgtgaaa   1320

ctgatgggta cttaactaaa atgacttgca gatggtctgc aaacccaaac gcattgctct   1380

tggggagttc cttgcagtta aaataccaca gcaaaattta ttgttctaac tttccaagta   1440

ctcctccaga atcagaggtg aaagaatgcc atttccagag gaatcattct tatgagtgca   1500

catttcagcc tgttttct ttatctggac ataccatgtg gattgagctt aagcactcgc   1560

tgggaacact tgaatcctca ccaacttgtg tcgttccagc agatgtggtg aagccactgc   1620

ctccctccaa cattaaagca gagatcacca gaaacgatgg gctgctgaac gtgagctgga   1680

caaaccccgt gtttacaaat gatgacctta gtttcagat ccggtgcgca gtgaacaggg   1740

aagaactcac atgggagctg tatgaagttc taagcgtacc aacaagatca gctgtgatag   1800

aagtgcaact ttgtgttgaa tatattgttc agatccgctg cagagccctg gatggcttag   1860

gctactggag caactggagc agatcagcct atgcagctgt aaaagatatc caagctccct   1920

tacatggccc tgagttttgg agaactgtca ctgaagatcc agcaacgggg cagaagaacg   1980

ttacgctcct gtggaagcca ctgatgaaga tcactcact gtgcagtgtg agccggtacg   2040

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ttggagcttc ttcagttaat tttaatttaa ctctgtcaca acaaatgagc acagtgaatg   2220

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aagaggagat gaagtggggtg caagttcctc caaatattag taaacactat atttatgacc   2400

actttattct gattgagaag taccggttca gcctgtaccc cgtgtttgct gcaggagttg   2460

gcaaatccag agccacggat cagttctcca agatgggta tgccagtcag accagttcta   2520
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9

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acctctatat ggtcctgcca atagttattt caacctccgt gctgttgctt ggagcgctgc   2580

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gctcgtgggc acaaggtgtt gattttcagc agcctgaaac tttttgagcac cttttttgtca   2700

agcaccctga agcaatgtca tttgagcctc ttcttctgga accggaaata gtgctggaag   2760

acatcagtgt tactaaagct ttggaacaag aagacacgca agatttctta gcgcttgatt   2820

ccatgttcac aaaacctgaa gactctgagc acgactctgc gtgtcccagc agccacttca   2880

gtggtcgcag ctccatggag tgctcccca gtgacccgac ctctggagaa acagcaagtc   2940

agtccaacat taagtatgcc actgttatca gtaactccag atcaggtggg ctttacgaac   3000

agaataagaa tcccagatgt cattttgatg gatgttttct agctgaagac tccttggctg   3060

caggtgcttg ctcaggtagc tcctgggagc tgggaaatga ggcgttcctc ctgctgcctg   3120

accagcctgg cagccagccc tgcaagaccc tctcccttat ctcttcagag ggattttccg   3180

agccttcaga tcaggatgat gctttcacag atggaggtag ccctgagcga ggtctccatt   3240

acctagggat aacatcactg ggcaaaagag aaaatgacat ttttttaaca gaaagttcca   3300

gactgatgtg ccatttccat acagctgatc tgctcagagg tgtgggattt cttcagaata   3360

cgcctcctaa tttaaatgca tttatccaga gtagcattaa agccatcgtg ccatacgtgc   3420

cgcagtttca gatgactgct gccaaggtgc aagagaccac agagaacagc tgttaagccc   3480

tcacacctta atttgatgct ctttatggct ttctaagtgt aggtatgtac tgtgtttttc   3540

cctctcctag cttctttaag gaaggttggg aagctgatcc tgtccttgag tgacaggatt   3600

ttagttggaa gaaattcccc ttcatagaat catagaactc tatgattcta tgatggccct   3660

tcytgaccta attttcaaat gtggggcctt cttttatata atttatataa aaatatattc   3720

aaaggatgtc tgttggccat gcagacccaa gcacgcgtct ccccattcag gagtaaagca   3780

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ggagcccaag                                                          3850
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<210> 28

<211> 1146

<212> PRT

<213> Gallus domesticus

<400> 28

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Met Tyr His Gln Ile Ile Leu Thr Met Ser Leu Leu Leu Gly Phe Leu
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His Val Ala Ala Ala His Cys Met Val His Glu Ile His Pro Arg Ser
            20                  25                  30

Phe Thr Leu Pro Cys Leu Leu Leu Asn Glu Thr Ser Ser Ser Pro Ser
            35                  40                  45

Ala Ala Gly Val Val Arg Ser Arg Ser Gly Trp Arg Arg Glu His Gly
        50                  55                  60
```

10

```
Val Ala Glu Thr Asn Pro Ser Leu Leu Met Asp Glu Glu Ser Phe Leu
65              70              75              80

Cys Cys Leu Trp Ser Asn Asn Asn Ala Ser Cys Ser Leu Tyr Ser Ala
            85              90              95

Asn Met Gln Ala Arg Met Leu Ile Pro Ser Glu Met Ser Ile Ser Ala
        100             105             110

Ser Gln Glu Arg Asp Ser Asn Trp Asn Ile Glu Cys Trp Val Glu Gly
        115             120             125

Lys Leu Asp Leu Leu Val Cys Ser Leu Gln Phe Pro Lys Phe His Met
    130             135             140

Arg Leu Asp Met Lys Val His Leu Leu Tyr Ala Val Ser Glu Leu Ser
145             150             155             160

Leu Gly Asp Thr Ser Thr Ser Thr Leu Lys Arg Thr Ala Leu Ala Ala
            165             170             175

Gln Cys Asn Cys Ser Glu Tyr Gly Lys Cys Glu Cys His Val Pro Ser
            180             185             190

Pro Arg Leu Asn His Thr Tyr Val Met Trp Leu Lys Thr Gly Ile Gly
        195             200             205

Leu Thr Pro Val Trp Ser Pro Leu Met Ser Ala Lys Pro Ile Asp Ile
    210             215             220

Val Lys Pro Asp Leu Pro Leu Asn Val Arg Leu Glu Met Thr Glu Arg
225             230             235             240

Gly Gln Val Lys Ile Cys Trp Ser Glu Pro Val Pro Met Pro Tyr Pro
            245             250             255

Leu Arg Cys Glu Val Asn Ile Phe Trp Lys Phe Arg Ser Asn Asp Trp
            260             265             270

Gln Gly Val Val Gln Val Ala Leu Asn Thr Ser Leu Asp Ile Asp Asn
            275             280             285

Met Leu Leu Asp Ser Ser Ser Phe Ala Gln Val Arg Cys Lys Ser Ile
    290             295             300

Cys Val Pro Gly Phe Trp Ser Glu Trp Ser Thr Leu Tyr Asn Leu Asn
305             310             315             320

Gly Glu Val Leu Tyr Phe Pro Thr Lys Ile Leu Thr Ser Val Gly Ser
            325             330             335

Asn Val Ser Phe His Cys Ile Tyr Lys Asn Lys Thr Gln Ser Val Ala
            340             345             350

Ser Lys Lys Ile Val Trp Trp Leu Asn Leu Ala Glu Glu Ile Pro Glu
        355             360             365

Ser Gln Tyr Thr Leu Val Asn Asp Arg Val Ser Lys Val Thr Leu Phe
    370             375             380

Asn Leu Lys Ala Thr Lys Pro Arg Gly Ser Phe Phe Tyr Asn Ala Leu
385             390             395             400

Tyr Cys Cys His Gln Asn Arg Glu Cys His His Arg Tyr Ala Glu Leu
            405             410             415

Tyr Val Val Asp Val Asn Ile Asn Ile Lys Cys Glu Thr Asp Gly Tyr
        420             425             430

Leu Thr Lys Met Thr Cys Arg Trp Ser Ala Asn Pro Asn Ala Leu Leu
```

11

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                435                   440                   445

Leu Gly Ser Ser Leu Gln Leu Lys Tyr His Ser Lys Ile Tyr Cys Ser
    450               455               460

Asn Phe Pro Ser Thr Pro Pro Glu Ser Glu Val Lys Glu Cys His Phe
465               470               475               480

Gln Arg Asn His Ser Tyr Glu Cys Thr Phe Gln Pro Val Phe Leu Leu
            485               490               495

Ser Gly His Thr Met Trp Ile Glu Leu Lys His Ser Leu Gly Thr Leu
            500               505               510

Glu Ser Ser Pro Thr Cys Val Val Pro Ala Asp Val Val Lys Pro Leu
        515               520               525

Pro Pro Ser Asn Ile Lys Ala Glu Ile Thr Arg Asn Asp Gly Leu Leu
    530               535               540

Asn Val Ser Trp Thr Asn Pro Val Phe Thr Asn Asp Asp Leu Lys Phe
545               550               555               560

Gln Ile Arg Cys Ala Val Asn Arg Glu Glu Leu Thr Trp Glu Leu Tyr
            565               570               575

Glu Val Leu Ser Val Pro Thr Arg Ser Ala Val Ile Glu Val Gln Leu
            580               585               590

Cys Val Glu Tyr Ile Val Gln Ile Arg Cys Arg Ala Leu Asp Gly Leu
        595               600               605

Gly Tyr Trp Ser Asn Trp Ser Arg Ser Ala Tyr Ala Ala Val Lys Asp
    610               615               620

Ile Gln Ala Pro Leu His Gly Pro Glu Phe Trp Arg Thr Val Thr Glu
625               630               635               640

Asp Pro Ala Thr Gly Gln Lys Asn Val Thr Leu Leu Trp Lys Pro Leu
            645               650               655

Met Lys Asn His Ser Leu Cys Ser Val Ser Arg Tyr Val Ile Lys His
            660               665               670

Gln Thr Ser Glu Asn Thr Ser Trp Ser Glu Tyr Val Asp Asn Gly Thr
        675               680               685

Thr Cys Ser Phe Pro Trp Thr Glu Ser Thr His Thr Ile Thr Phe Leu
    690               695               700

Ala Val Asn Ser Ile Gly Ala Ser Ser Val Asn Phe Asn Leu Thr Leu
705               710               715               720

Ser Gln Gln Met Ser Thr Val Asn Ala Val Gln Ser Leu Ile Ala Tyr
            725               730               735

Pro Val Asn Ser Thr Cys Val Ile Leu Thr Trp Thr Leu Ser Pro Gln
            740               745               750

Ile Tyr Val Ile Thr Ser Phe Ile Ile Glu Trp Arg Asn Leu Asn Lys
        755               760               765

Glu Glu Glu Met Lys Trp Val Gln Val Pro Pro Asn Ile Ser Lys His
    770               775               780

Tyr Ile Tyr Asp His Phe Ile Leu Ile Glu Lys Tyr Arg Phe Ser Leu
785               790               795               800

Tyr Pro Val Phe Ala Ala Gly Val Gly Lys Ser Arg Ala Thr Asp Gln
            805               810               815
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Phe Ser Lys Asp Gly Tyr Ala Ser Gln Thr Ser Ser Asn Leu Tyr Met
        820   825   830

Val Leu Pro Ile Val Ile Ser Thr Ser Val Leu Leu Leu Gly Ala Leu
        835   840   845

Leu Val Ser His Arg Arg Met Lys Lys Leu Leu Trp Glu Asp Val Pro
    850   855   860

Ile Pro Lys Asn Cys Ser Trp Ala Gln Gly Val Asp Phe Gln Gln Pro
865   870   875   880

Glu Thr Phe Glu His Leu Phe Val Lys His Pro Glu Ala Met Ser Phe
            885   890   895

Glu Pro Leu Leu Leu Glu Pro Glu Ile Val Leu Glu Asp Ile Ser Val
        900   905   910

Thr Lys Ala Leu Glu Gln Glu Asp Thr Gln Asp Phe Leu Ala Leu Asp
        915   920   925

Ser Met Phe Thr Lys Pro Glu Asp Ser Glu His Asp Ser Ala Cys Pro
    930   935   940

Ser Ser His Phe Ser Gly Arg Ser Ser Met Glu Cys Ser Pro Ser Asp
945   950   955   960

Pro Thr Ser Gly Glu Thr Ala Ser Gln Ser Asn Ile Lys Tyr Ala Thr
            965   970   975

Val Ile Ser Asn Ser Arg Ser Gly Gly Leu Tyr Glu Gln Asn Lys Asn
        980   985   990

Pro Arg Cys His Phe Asp Gly Cys Phe Leu Ala Glu Asp Ser Leu Ala
        995   1000   1005

Ala Gly Ala Cys Ser Gly Ser Ser Trp Glu Leu Gly Asn Glu Ala
    1010   1015   1020

Phe Leu Leu Leu Pro Asp Gln Pro Gly Ser Gln Pro Cys Lys Thr
    1025   1030   1035

Leu Ser Leu Ile Ser Ser Glu Gly Phe Ser Glu Pro Ser Asp Gln
    1040   1045   1050

Asp Asp Ala Phe Thr Asp Gly Gly Ser Pro Glu Arg Gly Leu His
    1055   1060   1065

Tyr Leu Gly Ile Thr Ser Leu Gly Lys Arg Glu Asn Asp Ile Phe
    1070   1075   1080

Leu Thr Glu Ser Ser Arg Leu Met Cys His Phe His Thr Ala Asp
    1085   1090   1095

Leu Leu Arg Gly Val Gly Phe Leu Gln Asn Thr Pro Pro Asn Leu
    1100   1105   1110

Asn Ala Phe Ile Gln Ser Ser Ile Lys Ala Ile Val Pro Tyr Val
    1115   1120   1125

Pro Gln Phe Gln Met Thr Ala Ala Lys Val Gln Glu Thr Thr Glu
    1130   1135   1140

Asn Ser Cys
    1145

<210>   29

<211>   18

13

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<212>   DNA

<213>   Synthetic Oligonucleotide


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<210>   30

<211>   24

<212>   DNA

<213>   Synthetic Oligonucleotide


<400>   30
cggcagcaga aggactcgaa gatg                             24


<210>   31

<211>   24

<212>   DNA

<213>   Synthetic Oligonucleotide


<400>   31
cacatggcac tcacatttgc cata                             24
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